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Original article

Assessing stingless bee pollen diet by analysis of garbage pellets: a new method

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Abstract – Studies on pollen resources by stingless bees frequently suffer from low sample size due to difficulties concerning the acquisition of harvested pollen. Here we describe a funnel-trap that allows non-invasive and automated sampling of pollen-rich garbage pellets that are expelled from colonies by workers bees. Single garbage pellets of *Trigona collina* from Sabah, Malaysia, contained between 7 and 11 different morphotypes of pollen and the similarity of the pollen composition of pellets expelled by a given colony on a given day was very high (quantitative Steinhaus index: 71 to 90%). The turn-over of pollen types in samples taken at consecutive points in time was relatively low over periods of three weeks (52 to 75% similarity) and variable over periods of four to six months (13.6 to 58.5% similarity). The comparison of pollen in corbicular loads and garbage pellets indicates that garbage pollen is derived from both feces of pollen-consuming workers and larval feces (meconia). The slow turn-over of pollen in garbage suggests that sampling at relatively long intervals (4–6 months) will be sufficient for a crude assessment of long-term pollen resources of stingless bee colonies.

pollen foraging / resource use / pollen trap / feces / fecal pellets

1. INTRODUCTION

Pollen is the principal source of nitrogen for most stingless bees and is collected in large quantities by workers for provisioning brood cells or for storage in colony pollen pots (Roubik, 1989). Palynological methods allow detailed analyses of pollen samples (Biesmeijer and Sommeijer, 1992), and pollen diets of stingless bee colonies have been investigated in several studies of floral resource use and partitioning (Engel

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and Dingemans-Bakels, 1980; Sommeijer et al., 1983; Absy et al., 1984; Appanah et al., 1986; Roubik et al., 1986; Ramalho et al., 1989; Lobreau-Callen et al., 1990; Ramalho, 1990; Nagamitsu et al., 1999; Roubik and Moreno, 2000; Eltz et al., in press). To obtain pollen samples these authors either collected corbicular pollen directly from incoming workers or, more frequently, extracted pollen from colony storage pots or deposits in the nest. The first method is generally time-consuming and requires frequent sampling, and the second often suffers from lack of accessible colonies. Therefore, low or unequal sample size and/or lack of sufficient colony replicates have been problematic in studies of stingless bee pollen diet.

Alternative methods of sample acquisition are required that can be applied to reasonable numbers of wild colonies of bees. Unfortunately, permanent pollen traps similar to those used for honeybees (Imdorf, 1983; Imdorf and Wille, 1983) are of little use in case of meliponines due to their tendency to use resin to obstruct any device imposed on their nest entrances. Many stingless bees, however, expel from their nests small parcels of garbage that have been reported to contain larval feces (Roubik, 1989; Roubik, pers. comm.). This garbage, potentially rich in pollen exines, is either directly dropped from the nest entrance or, more frequently, scattered in the vicinity of the nest by airborne workers (Roubik, 1989; T. Eltz, pers. obs.). It is the aim of this article to (i) introduce an efficient way of trapping garbage from wild colonies of stingless bees in Malaysia, and (ii) to investigate whether and how pollen obtained from garbage samples can be used to indicate bee pollen diet.

2. MATERIALS AND METHODS

Bee garbage was collected from stingless bee nests situated in the bases of large trees in lowland mixed-dipterocarp forest in Sabah, Malaysia. Our study focused on *Trigona (Tetragonula) collina*, an abundant, medium-sized species in the study area. Unless stated otherwise, all information given concerns this species.

2.1. The garbage trap

Garbage pellets were sampled using funnel traps that were installed in front of nest entrances. Traps consisted of (i) a transparent funnel, (ii) a garbage sieve made of pins suspended by a double layer of nylon mesh within a PVC tube, and (iii) a sampling jar (Fig. 1). Bees exiting the nest inevitably enter the transparent funnel, crawl towards

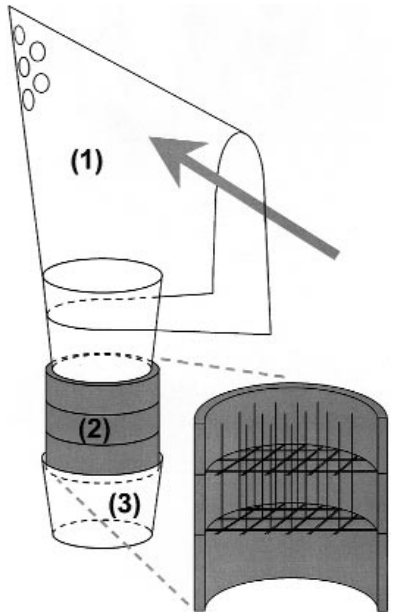

Figure 1. Schematic view of the garbage trap: (1) Transparent funnel made from clear DIN A4 overhead transparency, (2) garbage sieve (grey) that consists of a dense array of household pins suspended in PVC tubing by a double layer of nylon mesh (mesh width ~0.5 cm), and (3) sampling jar. The trap is placed in front of the nest entrance and exiting bees enter the funnel as indicated by the arrow. Bees can escape from the funnel through punctures in the tip (diameter ~3 mm) unless carrying garbage pellets (see text for further specifications).

the tip guided by increasing light levels and finally leave the funnel through one of a series of holes (diameter ~3 mm) punched into the tip area. In the case of bees carrying garbage pellets in their mandibles, the holes were too small to serve as escape routes and the bees instead sought to circumvent the trap. In attempting to do so, most of the individuals sooner or later fell onto the pin cushion of the garbage sieve and, unable to comfortably walk on the spaced pins (distance ~0.5 cm), released their garbage loads. The pellets dropped through the sieve and into the sampling jar. In the course of one day a trap collected between a dozen and several hundred garbage pellets, depending on colony size and status (Eltz, pers obs.). In our study, pellets were placed singly or in tens (pooled samples) in Eppendorf cups and frozen until further analysis.

2.2. Macroscopic examination

For general description, garbage pellet contents were first studied by dissecting single pellets (dissolved in water or 70% EtOH) under a stereomicroscope. In addition to pellets from *T. collina*, we also examined samples obtained from colonies of the following species: *T. (Tetr.) melanocephala*, *T. (Tetr.) melina*, *T. (Lepidotrigona) terminata*, and *T. (Tetrigona) binghami*.

2.3. Pollen content

To obtain a quantitative estimation of total pollen content of pellets, we used acetolysis for pollen concentration. Twelve pooled samples of ten pellets each, collected from twelve different colonies of *T. collina*, were dried for 24 h at 60 °C, weighed, and consecutively acetolysed (Moore et al., 1991). Acetolysis removes all but the chemically most resistant components and reduces garbage pellets to sediments of almost pure pollen exines. The proportional weight of the treated sample (after drying, in %) can therefore be used as a conservative but standardized measure of garbage pollen content.

2.4. Microscopic pollen analysis

For a more detailed analysis of pollen type composition of garbage of *T. collina* we investigated the following sets of samples:

1. Single-pellet garbage samples taken from two colonies (colony A and B) at Deramakot Forest Reserve at three consecutive points in time separated by approximately three weeks (10 single-pellet samples for each colony on each of April 10, May 2, and May 25 in 1999). The colonies were located in secondary forest at two different sites separated by 8 km and almost certainly had non-overlapping flight ranges (see van Nieuwstadt and Ruano Iraheta, 1996).

2. Ten-pellet (pooled) garbage samples taken from one of the colonies (colony B) at four points in time separated by four to six months (one pooled sample in April 1999, September 1999, March 2000, and July 2000).

3. Ten-pellet (pooled) garbage samples and corbicular pollen samples taken from a colony (colony C) at Sepilok Forest Reserve in intervals of 12 days (average) between February 27 and July 23 in 2000. In this case we used a modified version of the trap that maximized sampling of corbicular pollen loads from incoming pollen foragers in addition to outbound garbage pellets. This was achieved by increasing the diameter of the sampling jar in order to capture corbicular pollen that was stripped off worker bees attempting to enter through the punctured funnel from the outside (most of the loads drop off on the outside rather than the inside of the funnel wall). Due to the fact that garbage pellets and corbicular pollen loads are quite compacted and stable it was possible to collect both items separately for each consecutive sample. Cross-contamination of pollen between corbicular loads

and garbage pellets probably occurs but was considered to be low enough to be eliminated by the 0.5%-volume threshold applied for analysis (see below). All corbicular loads of one sampling day were pooled. Even the modified traps do not normally collect large quantities of corbicular pollen and substantial amounts were obtained only because colony C was exceptionally populous.

Standard palynological protocols (KOH digestion, acetolysis, glycerin jelly mounting) were followed for slide making. The slides were analyzed in a standardized way. First, the core area of each slide was thoroughly searched for pollen types and types were characterized by size, shape, number and shape of the apertures, and ornamentation. Digital images were made from polar and equatorial views of all pollen types and images were entered into a pollen database that served as a working reference. Taxonomic identifications of types to the level of plant family, genus or species (or taxonomic 'type') were made from original slides by S. van der Kaars, partly by comparison with reference pollen collected from flowers in the bees' habitat.

For assessing pollen type representation on a slide we used a volume-based approach similar to Biesmeijer and Sommeijer (1992) that corrected for the huge size differences between different pollen species (the volume and, presumably, the amount of digestible protoplasm can vary over more than five orders of magnitude). A type-specific volume was assigned to each pollen type based on size and shape of the respective grain (geometric formulas for sphere or elipsoid volume were used for calculations). Grains were then counted in quadrants situated along transects across the center of the slides and counts/type/quadrant were immediately typed into Excel spreadsheets that continuously calculated cumulative volume for each type as well as total volume of pollen counted. In previous tests we found that sampling a total pollen volume of 6×10^6 cubic microns per slide would reflect pollen slide diversity with reasonable accuracy (concerning both type diversity and percentage representation). Thus, for standardized comparisons of pollen contents between slides we invariably stopped counting grains as soon as the total volume of grains counted exceeded 6×10^6 cubic microns. Grains represented with less than 0.5 volume-% were considered as contaminations and omitted from further analysis.

For comparisons of pollen composition between samples we calculated the Steinhaus coefficient S (Legendre and Legendre, 1998), with $S = 2W/(A + B)$, where W is the sum of minimum percentages of the various types, and A and B are the sums of the percentages of all types in each of two samples. Note that differences between dominant types reduce S to the same extent as do differences between minor types. For convenience we multiplied S with 100 to present values reflecting *percentage similarity*.

To estimate pollen type saturation in increasing numbers of garbage pellets sampled on a given day, we plotted type accumulation curves using the software Estimate S 5.0.1 by Robert K. Colwell.

3. RESULTS

3.1. General description of garbage pellets

Garbage pellets of *T. collina* are dark brown, roughly spherical particles of about 2 mm in diameter that have a conglomerate appearance. When dissected in water or EtOH almost all pellets reveal large quantities of pollen grains that make up most of the pellet volume. Other contents include the remains of old brood cells as well as parts of dead bees or parts of bee larvae and pupae. The spherical shape and the compactness of the pellet is maintained by layers and threads of resinuous material that are worked around and into the pellet. Due

to these inclusions the garbage pellets are quite stable items that are normally trapped intact and can easily be counted and preserved.

Garbage pellets of *T. melanocephala*, *T. melina*, *T. terminata* and *T. binhami* also contained large quantities of pollen and differed from those of *T. collina* only in respect to pellet size (depending on bee size) and, partly, color.

3.2. Pollen content

Mean dry weight of pooled ten-pellet garbage samples was 25.4 mg (± 4.6 mg; $N = 12$). After acetolysis treatment this was reduced to 5.2 mg (± 1.4 mg), thus indicating a content of pure pollen exines of 20.3% (± 1.5 %) of the dry weight. This value certainly underestimated the true pollen content of garbage pellets because acetolysis does dissolve the less resistant parts of pollen grains such as remaining cellular contents, the pollen kit and also parts of the pollen wall (Moore et al., 1991).

3.3. Pollen composition of single pellets

Microscopic slides made from garbage samples were very clear, almost devoid of non-pollen material, and invariably showed well preserved and evenly spread assemblages of pollen grains. Single garbage pellets of *T. collina* (colonies A and B) contained between seven and 11 different morphotypes of pollen above the 0.5%-volume threshold (Fig. 2). Volume representations were strongly skewed towards dominant grains, and between 84 and 96% of the sample volume consisted of the four most dominant grains of the sample.

Pellets collected on the same day from one colony were very similar in pollen composition, with mean percentage similarities ($S \times 100$) between pairs ranging from 71 to 90% (mean: 80.1 ± 8). This high degree of similarity is also reflected by the type accumulation curves shown in Figure 3. Single garbage pellets already contained between 60 and 80% of the pollen types found in the entire set of samples taken from one nest on a given day (10 to 15 types total), and all but one set of samples (nest B, May 2) showed rapid type saturation within the observed range of replicates.

3.4. Temporal variation of pollen composition

The pollen composition of single garbage pellets collected at different times in April and May 1999 is shown in Figure 2. Although some differences are apparent between sampling dates in both nests, the similarities were still high. Average percentage similarities between samples collected on consecutive dates are given in Table I. Many of the dominant pollen types (e.g. Leguminosae A, *Durio* type, *Convolvulus* type, Leguminosae B) were present in variable quantities at all times in one or both colonies. In contrast, very few major types were restricted to one particular point in time (e.g. Leguminosae C).

This pattern partly changed when samples were separated by more than a few weeks. The pollen composition of pooled ten-pellet samples collected from colony B at intervals of 4 to 6 months in 1999 and 2000 is given in Table II. Each sample contained between 11 and 15 different types, summing to a total of 28. Similarity was low between samples of consecutive points in time in two cases (14.0% between April and September 1999; 13.6% between September 1999 and March 2000) and relatively high between March and July 2000 (58.5%). Although total similarity over time was partly low, a reasonable number of types (*Durio* type, *Manihot esculenta*, Leguminosae B, Leguminosae? A, Bombacaceae A) was found in substantial quantities at two or three points in time (Tab. II).

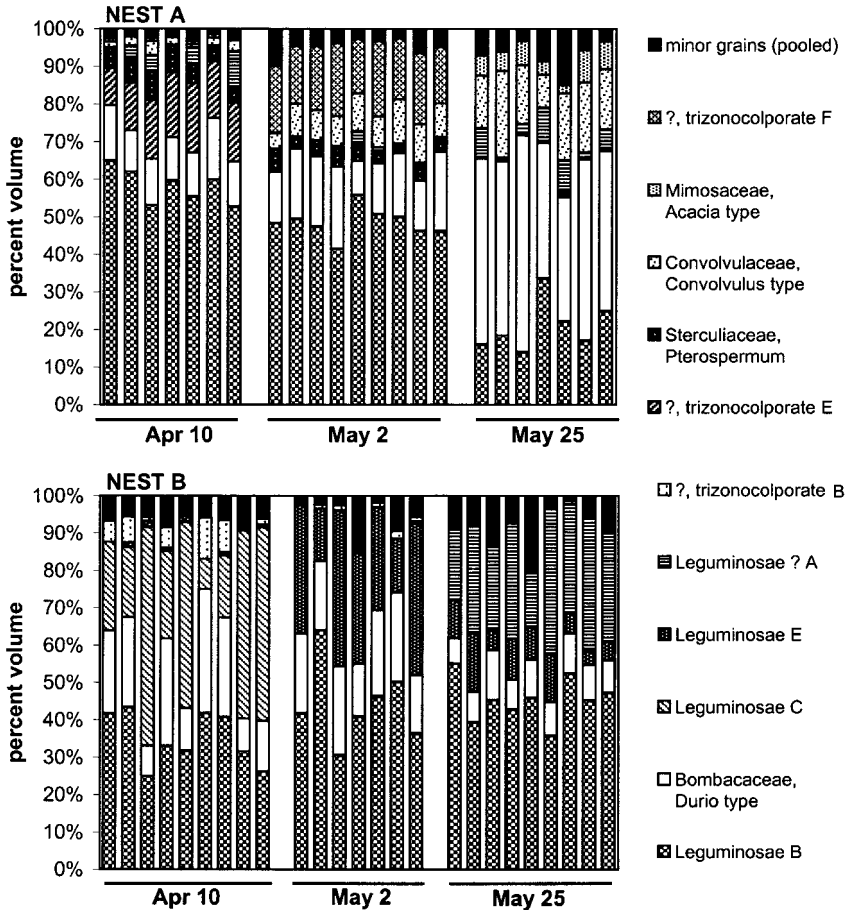

Figure 2. Pollen composition of single garbage pellets taken from two different colonies of *T. collina* (nest A and B, both in Deramakot Forest Reserve) at three different points in time in 1999. Only dominant pollen types are shown in detail.

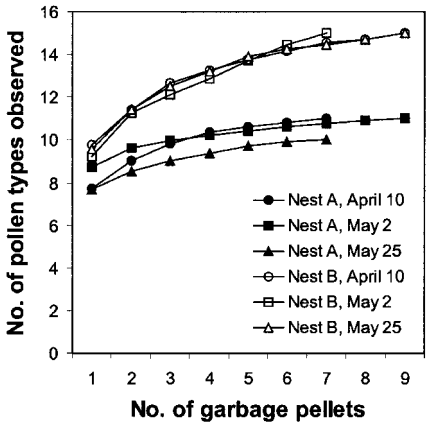

Figure 3. Pollen type accumulation in samples of garbage of two colonies of *T. collina* (Nest A and B) collected at three different points in time in 1999. Note the rapid type saturation after adding relatively small numbers of garbage pellets. Curve parameters were calculated with Estimate S 5.0.1 software using 50 randomizations.

Table I. Similarity of pollen composition of single garbage pellets taken at consecutive points in time from a given colony of *T. collina* (for colonies A and B). Values are Steinhaus-Index × 100 and represent percentage similarity. All possible pairwise comparisons between single pellets were included and means were calculated across these comparisons.

Colony	Dates compared	Number of pellets and pairwise comparisons	Mean %	Minimum %	Maximum %
			similarity		
A	April 10 with May 2	7 × 9 = 63	75.4	67.8	83.1
A	May 2 with May 25	7 × 9 = 63	52.0	36.0	71.7
A	April 10 with May 25	7 × 7 = 49	38.9	27.8	58.2
B	April 10 with May 2	7 × 7 = 49	52.8	36.3	72.1
B	May 2 with May 25	7 × 7 = 49	60.1	45.8	71.7
B	April 10 with May 25	9 × 9 = 81	46.4	34.9	59.5

Table II. Pollen composition of pooled ten-pellet garbage samples taken from *T. collina* colony B at intervals of 4 to 6 months in 1999 and 2000 (Deramakot Forest Reserve). Values are percent volume of the entire sample.

No.	Pollen type/species	April	September	March	July	Mean
		1999		2000		
1	Leguminosae? A		7.80	49.80	26.97	21.14
2	Rubiaceae, *Neonauclea* type?		60.46	1.86		15.58
3	Leguminosae B	34.10	5.73			9.96
4	Bombacaceae A			22.89	15.19	9.52
5	Leguminosae C	32.96				8.24
6	Bombacaceae, *Durio* type	18.95	5.38		2.88	6.80
7	Euphorbiaceae, *Manihot esculenta*			6.02	18.57	6.15
8	Mimosaceae, *Mimosa pudica* type	0.56	1.35	5.83	7.08	3.70
9	Oleaceae. *Jasminium* type	0.84			10.91	2.94
10	?, trizonocolporate A	1.08	6.47	0.84	1.51	2.47
11	Asteraceae, Tubulifloreae A type		0.53	6.60	1.92	2.26
12	Leguminosae D		6.86			1.72
13	Leguminosae E	0.86		0.79	4.90	1.64
14	Symplocaceae, *Symplocos* type		0.65		5.15	1.45
15	Passifloraceae, *Passiflora*?	1.29	1.12	1.84	0.63	1.22
16	?, trizonocolporate B	4.35				1.09
17	Caesalpiniaceae, *Caesalpinia* type			2.49		0.62
18	Bombacaceae B		2.19			0.55
19	Combretaceae, *Terminalia* type	1.70			0.42	
20	Leguminosae? F	1.46			0.36	
21	?, trizonocolporate C				1.21	0.30
22	Rutaceae, *Clausena* type				1.02	0.25
23	?			0.69		0.17
24	?, trizonocolpate				0.68	0.17
25	?, trizonocolporate D	0.61				0.15
26	Euphorbiaceae, *Croton* type				0.59	0.15
27	Rutaceae, *Citrus* type	0.51				0.13
28	Sterculiaceae, *Sterculia* type		0.50			0.13
	No. of types/sample	13	12	11	15	28

3.5. Comparison between corbicular and garbage pollen

The modified trap collected sufficient amounts of incoming corbicular pollen to allow comparisons with garbage pollen. Between 15 and 136 (mean: 52.7; $N = 13$) corbicular loads were counted on any sampling day, with pollen type diversity being dependent on the number of loads in pooled samples (Fig. 4) (Nonlinear Regression, S model: $R^2 = 0.61$; $F = 15.59$; $p < 0.01$). The shape of the saturating curve suggests that the daily pollen import of colony C at the time of the study comprised about ten different types.

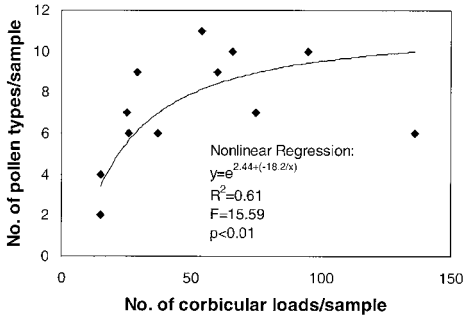

Figure 4. Relationship between the number of corbicular loads per pollen sample and type diversity. A non-linear S model produced the best fit among models offered by the SPSS statistical package. The model saturates at 11.4 pollen types for very large load numbers.

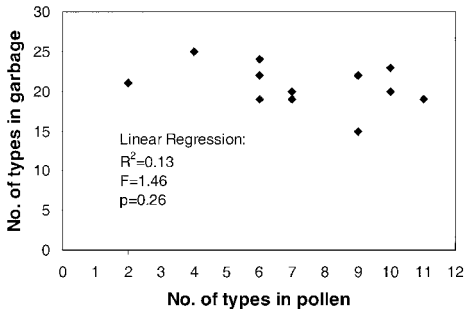

Figure 5. Relationship between the number of pollen types in garbage samples and that in corbicular samples collected at the same day. No effect was observed.

The number of types found in samples of garbage at the same time was twice as high on average (20.8 ± 2.61), and there was no relationship between the number of types found in corbicular load samples and garbage samples at a given day (Fig. 5). Almost all (94%) of the types that were collected as pollen (total: 32) did also appear in the garbage samples but only 65% of the types found in garbage samples (total: 46) were also detected in pollen loads during the study period. Generally garbage pollen diversity was much higher in colony C than in colony A and B from Deramakot Forest Reserve (see above).

Figure 6 shows the phenology of major pollen types over time in samples of garbage as well as corbicular pollen. Although patterns varied, the data allow two generalizations: (i) Representation of types in corbicular load samples was usually much more restricted in time than representation in garbage, and (ii) representation in garbage tends to peak at times when types were also present in corbicular loads. In some cases major grains were present in garbage samples long before (e.g. *Zea mays*, Bombacaceae A) or after (Mimosaceae? type) any sign of import of those types was apparent in the corbicular load samples, and in one case (Cucurbitaceae type) a major grain was not present in corbicular loads at all.

4. DISCUSSION

Our results show that garbage pellets of *T. collina* contain large quantities of pollen. Although the origin of pollen in garbage has not been directly demonstrated by our study it is very likely that it is derived from feces previously deposited in the nest by bee larvae and adults. Pollen exines have been shown to remain largely unharmed by digestion (Stanley and Linskens, 1974; Klungness and

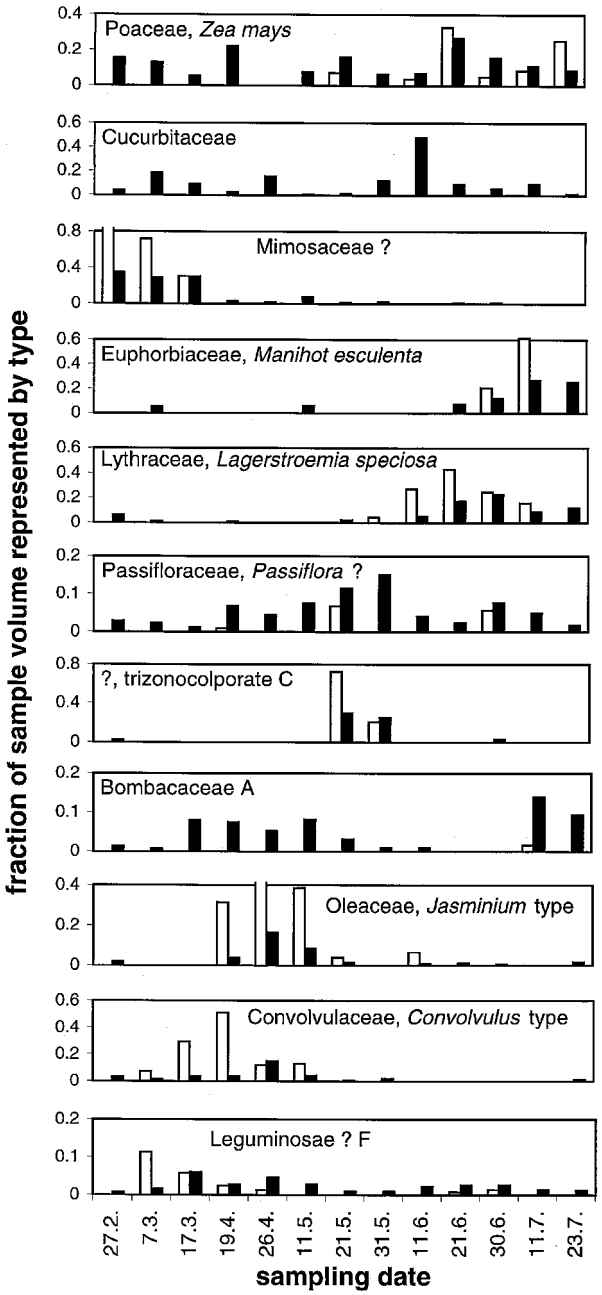

Figure 6. Temporal distribution of major pollen types in samples of garbage (black bars) and corbicular pollen (white bars) taken from colony C between February and July in 1999.

Peng, 1984; Crailsheim et al., 1992), a finding that can explain the high degree of preservation of garbage pollen.

4.1. Dynamics of garbage pollen composition

Temporal patterns of pollen type representation in samples of garbage and corbicular pollen are varied and suggest that several factors interact in determining when foraged pollen is likely to appear in garbage pellets. The processes involved occurred over a range of different time scales and are likely to include (i) defecation of pollen directly consumed by workers, (ii) inclusion of fecal pellets (meconia) removed from brood cells after a lag-time of several weeks for larval development, and (iii) consumption and defecation of pollen from long-term storage pots. The flow of pollen in stingless bee colonies can be complex and has been studied in detail by Sommeijer et al. (1985) for *Melipona favosa*. After being deposited in pollen pots by foragers, pollen is first ingested by workers of a wide range of ages which then pass it to others in liquid form (trophallaxis). The flow is finally directed to a limited number of younger workers that eventually provision empty brood cells (Sommeijer et al., 1985). It is unknown how much of the pollen is actually digested and consumed by adult workers during the process of trophallaxis, but the amounts could be substantial. We believe that direct pollen consumption and defecation by workers in the nest is responsible for our finding that some pollen types synchronously peaked in both garbage and corbicular pollen samples. In contrast, inclusion of larval feces and consumption of pollen from long-term storage is likely to lead to the pronounced time-lags as evidenced in some dominant pollen types. Generally, the temporal dynamics of pollen appearing in garbage is likely to depend on the nutritive state of the colony and may therefore vary considerably over time. Consequently, garbage trapping cannot easily be used for studies of short-term temporal dynamics of pollen foraging. On a time scale of weeks there will be no straightforward relationship between the time of collection of certain pollen species and their appearance in garbage samples. However, if long-term patterns of resource use are of interest, repeated garbage trapping can be used for measuring pollen turnover over seasons and years.

4.2. Sampling efficiency

Pollen contents of garbage pellets collected at one point in time from a given colony were surprisingly similar in type composition and relative abundance. This implies that sampling small numbers of pellets will provide a good estimate of garbage pollen composition at the time. Moreover, the turn-over of garbage pollen was low, presumably because the processes described under 4.1 are integrating harvested pollen over a range of time scales. Thus, it seems that sampling at relatively long intervals (4–6 months as for colony B in this study) will be sufficient for a crude assessment of long-term colony pollen diet.

So far garbage samples have been collected from colonies of five species of stingless bees in Malaysia, but the method is likely to be applicable to a much wider range of meliponines (see Roubik, 1989). Non-invasive sampling of garbage pollen will prove especially advantageous in studies on natural bee populations and communities because of the fact that it is not necessary to fell entire nest trees in order to access colony pollen stores. Therefore, and because the described garbage traps allow automated synchronous sampling from large numbers of colonies, the analysis of garbage pollen should be useful in future studies of stingless bee resource use, interspecific resource partitioning or competition.

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Résumé – Une nouvelle méthode pour estimer le régime pollinique des abeilles sans aiguillon par l'analyse des pelotes de déchets. Les méthodes actuelles pour estimer le régime pollinique des abeilles sans aiguillon impliquent soit un lourd travail pour échantillonner le pollen des corbicules des butineuses qui rentrent, soit l'accès direct aux réserves polliniques de la colonie. Les études sur l'utilisation des ressources polliniques ont donc souvent pâti d'un faible échantillonnage. Nous décrivons ici un piège qui permet un échantillonnage automatique et non invasif des pelotes de déchets, parcelles de déchets que les ouvrières tiennent entre leurs mandibules et qu'elles expulsent hors de la colonie. Le piège comporte (i) un entonnoir transparent, (ii) un tamis à déchets, constitué de rangées d'épingles et (iii) un collecteur (Fig. 1) ; il est placé devant l'entrée du nid. Les ouvrières qui sortent pénètrent dans l'entonnoir (1, sens de la flèche), volent vers la lumière et passent à travers les orifices percés à la pointe de l'entonnoir. Ceci est impossible si elles portent des pelotes de déchets. Elles sont alors obligées de laisser tomber leur charge à travers le tamis (2) dans le collecteur (3).

Dans le cas de la principale espèce étudiée, *Trigona (Tetragonula) collina*, qui vit dans les forêts de plaine de Sabah (Malaisie), 20 % du poids sec des pelotes de déchets ont des exines bien conservées qui peuvent être analysées au microscope. Les pelotes de deux colonies (A et B, Fig. 2) de *T. collina* contenaient entre 7 et 11 morphotypes polliniques et la composition pollinique des pelotes expulsées par une colonie donnée à un jour donné a très peu varié (index quantitatif de Steinhaus : 71 à 90 %). Le renouvellement dans le temps des types polliniques a été faible : les échantillons prélevés à intervalles de trois semaines présentaient une forte similitude (52 à 75 %) ; ceux prélevés à intervalles de quatre à six mois avaient une similitude plus faible et variable (13,6 à 58,6 %). Dans une colonie de *T. collina* (colonie C, Fig. 6), nous avons comparé sur une période de cinq mois le pollen des pelotes de détritus et celui des pelotes de corbicules des ouvrières qui rentraient, en prélevant des échantillons des deux types tous les 15 jours. 94 % des types polliniques trouvés dans le pollen de corbicules étaient également présents dans le pollen des déchets, mais les échantillons de déchets contenaient d'autres types absents des pelotes de corbicules au moment de l'étude. La distribution dans le temps des types polliniques présents dans les pelotes de corbicules et les pelotes de déchets suggère que la composition pollinique des déchets est influencée par plusieurs processus qui agissent à des échelles de temps différentes. Les déchets comprennent : (i) la défécation du pollen directement consommé par les ouvrières, (ii) l'inclusion des excréments (meconia) éliminés des cellules de couvain après une période de latence de plusieurs semaines pour le développement larvaire et (iii) la consommation et la défécation du pollen issu des pots de stockage. Nous pensons que l'échantillonnage du pollen des déchets combiné à l'analyse microscopique est une méthode prometteuse pour estimer les régimes polliniques des abeilles sans aiguillon. En raison du lent renouvellement du pollen des déchets dans le temps et du fait que ce renouvellement est influencé par un éventail de processus qui interagissent, la méthode est surtout adaptée pour mesurer les modes d'utilisation du pollen sur le long terme. Ce lent renouvellement implique aussi qu'un échantillonnage à intervalles

longs (jusqu'à quatre à six mois) suffit pour estimer grossièrement les ressources polliniques d'une colonie.

butinage / pollen / utilisation des ressources / piège à pollen / fécès / pelote fécale

Zusammenfassung – Die Analyse von Pollen im Müllauswurf: Eine neue Methode zur Erfassung der Pollennahrung von Stachellosen Bienen. Die zur Zeit gebräuchlichen Methoden zur Erfassung der Pollennahrung von Stachellosen Bienen sind mit Schwierigkeiten verbunden, da sie entweder sehr arbeitsaufwendig sind (wie das Absammeln der Pollenhöschen von heimkehrenden Bienen) oder den direkten Zugang zu den Pollenvorräten der Kolonien voraussetzen. Aus diesen Gründen leiden viele der relevanten Untersuchungen unter zu geringen Stichprobengrößen. In dem vorliegenden Artikel beschreiben wir eine einfache Falle, mit deren Hilfe es gelingt, die von Arbeiterbienen aus dem Nest geworfenen, pollenhaltigen Müllballen aufzufangen. Diese Müllfalle, die aus (i) einem transparenten Trichter, (ii) einer Müllreuse und (iii) einem Auffanggefäß besteht (Abb. 1), wird vor der Eingangsröhre des Bienennestes aufgestellt. Arbeiterbienen fliegen zum Licht hin in den transparenten Trichter auf und können diesen normalerweise durch kleine Löcher in der Spitze verlassen. Mit Müllballen beladen ist dies jedoch nicht möglich und die Bienen werden schließlich gezwungen ihre Last durch die Reuse in einen Sammelbehälter fallenzulassen. Im Falle unserer hauptsächlichen Untersuchungsart, *Trigona (Tetragonula) collina* aus dem Tiefland von Sabah (Malaysia), bestehen etwa 20 % des Trockengewichts der Müllballen aus gut erhaltenen Pollenexinen, die als Grundlage für eine mikroskopische Pollenuntersuchung dienen können. Einzelne Müllballen von zwei Untersuchungskolonien von *T. collina* enthielten zwischen 7 und 11 verschiedene Pollen-Morphotypen. Die Pollenzusammensetzung von Ballen, die am selben Tag von einer Kolonie abgesammelt wurden, war sehr hoch (71–90 %, quantitativer Steinhaus-Index). Die zeitliche Dynamik von Pollen in Müllproben war niedrig: Bei Sammelintervallen von drei Wochen war die Ähnlichkeit aufeinander folgender Proben hoch (52–75 %), bei Intervallen von vier bis sechs Monaten erwartungsgemäß niedriger und in stärkerem Maße variabel (13,6–58,5 %). Exemplarisch verglichen wir bei einer Kolonie von *T. collina* über einen Zeitraum von fünf Monaten den Pollen-Eintrag (corbicularer Pollen) mit dem Müllpollen-Auswurf. 94 % der eingetragenen Pollentypen wurden auch im Müll gefunden, aber der Müll enthielt zusätzlich weitere Typen, die zur Untersuchungszeit nicht nachweislich eingetragen wurden. Die zeitliche Verteilung verschiedener Pollentypen in Eintrag und Auswurf legt nahe, daß die Zusammensetzung des Müllpollens von mehreren Prozessen beeinflußt wird, die auf unterschiedlichen Zeitskalen wirken: Bienenmüll enthält (i) Pollen, der direkt von Arbeiterbienen verzehrt und wieder ausgeschieden wird (bzw. dessen Exinen), (ii) Pollen aus den Exkrementen (Meconiae) der Larven, und (iii) Pollen, der nach Einlagerung in Pollenbehältern nach geraumer Zeit konsumiert und schließlich ausgeschieden wird.

Die beschriebene Müllfalle könnte sich in zukünftigen Studien als ein nützliches Hilfsmittel zur Erfassung der Pollenressourcen von Meliponinen erweisen. Aufgrund der langsamen zeitlichen Dynamik der Müllpollen-Inhalte und der Tatsache, daß verschiedene Prozesse an deren Zustandekommen beteiligt sind, ist die Methode hauptsächlich zur Erfassung langfristiger zeitlicher Muster der Ressourcennutzung geeignet. Der geringe Turn-over impliziert auch, daß für eine grobe, langfristige Erfassung der Pollenressourcen bereits Probennahmen in mehrmonatigen Abständen (bis zu 4–6 Monaten) ausreichend sind.

Pollenfalle / Müllballen / Blütenspektrum / Bienentracht / Pollenspektrum

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