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Research article

Collection of mold (*Rhizopus* sp.) spores in lieu of pollen by the stingless bee *Trigona collina*

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Summary. In the course of a study on pollen diets of three sympatric species of stingless bees (Apidae: Meliponini) in Sabah, Malaysia, we made the observation that large fractions of the foragers of three colonies of *Trigona collina* collected corbicular loads of fungal spores in lieu of pollen. Collection of spores continued for at least three consecutive days. The spores were brought to germination in the laboratory and the culture was identified as mold of the genus *Rhizopus* (Zygomycota, Mucorales). Our observations represent the first reported case of the collection of *Rhizopus* mold spores in lieu of pollen by bees and a rare case of the collection of fungal spores by bees other than honeybees (*Apis*).

Key words: Spore collection, pollen foraging, pollen diet, brood provisions, Meliponini

Pollen is the principal source of nitrogen for most bees and is normally collected in quantities in order to provision brood cells. The occasional collection of fungal spores in lieu of pollen by honeybees of the genus *Apis* has been reported in a fair number of cases all over the world. The fungi concerned were either rust fungi (Basidiomycota), powdery mildews or members of *Neurospora* (Ascomycota) (Shaw and Robertson, 1980; Wingfield et al., 1989; Shaw, 1990 and references therein). Very few observations have been reported on spore feeding by bees other than honeybees. Stingless bee workers (*Trigona* spp.) have been seen foraging on the slimy, spore-containing gleba of stinkhorns (Phallales) (Roubik, 1989; Burr et al., 1996; Oliveira and Morato, 2000), and in at least one case the exudates were actually stored in the corbiculae of the hind legs, suggesting that they may have been collected for provisioning brood cells (Burr et al., 1996). In southeast Brasil, G. Acevedo (cited as pers. comm. in Oliveira and Morato, 2000) observed workers of *Partamona helleri* (Friese, 1900) carrying corbicular loads of unidentified spores to the nest. Generally, data on the performance of brood raised on fungal spores is scant and it remains controversial whether the bees actually benefit from spore collection. In some cases, however, the sheer quantity of spores collected as well as the fact that spore collection takes place over considerable periods of time suggests that some nutritional benefit is likely to be obtained (Shaw, 1990).

Here we report on observations made on homing foragers of the stingless bee *Trigona collina* Smith (Apidae: Meliponini) in lowland rain forest in Sabah, Malaysia. Our findings are notable in two respects: first, they involve extensive fungal spore collection by bees other than honeybees, and second, the source fungus is a mold of the Zygomycota (Mucorales) that, to our knowledge, have never been shown to be of interest to foraging bees.

Our observations were made in the context of a study aimed at assessing pollen diets of six colonies of meliponines belonging to three species (*Trigona collina* (4 colonies), *Trigona melina* Gribodo (1), *Trigona melanocephala* Gribodo (1)) in selectively logged forest in Deramakot Forest Reserve (Eltz et al., 2001). To assess pollen diets we took repeated biweekly samples of corbicular loads of 20 homing foragers between March and May 1998. For this purpose we briefly closed the nest entrances and captured pollen foragers with the help of hand-held exhaustors. When collecting samples on May 7 we noted that substantial numbers of foragers of three of the four colonies of *T. collina* were carrying large corbicular loads of a fluffy black smear that could easily be distinguished from 'regular' pollen loads that were more compact and either white, yellow or orange. On May 7 12.5%, 25%, and 50% of the collected samples were of the black type for the three different colonies of *T. collina*. Although no quantitative observations were made, the continued collection of the black smear was confirmed for all three colonies on the two following days.

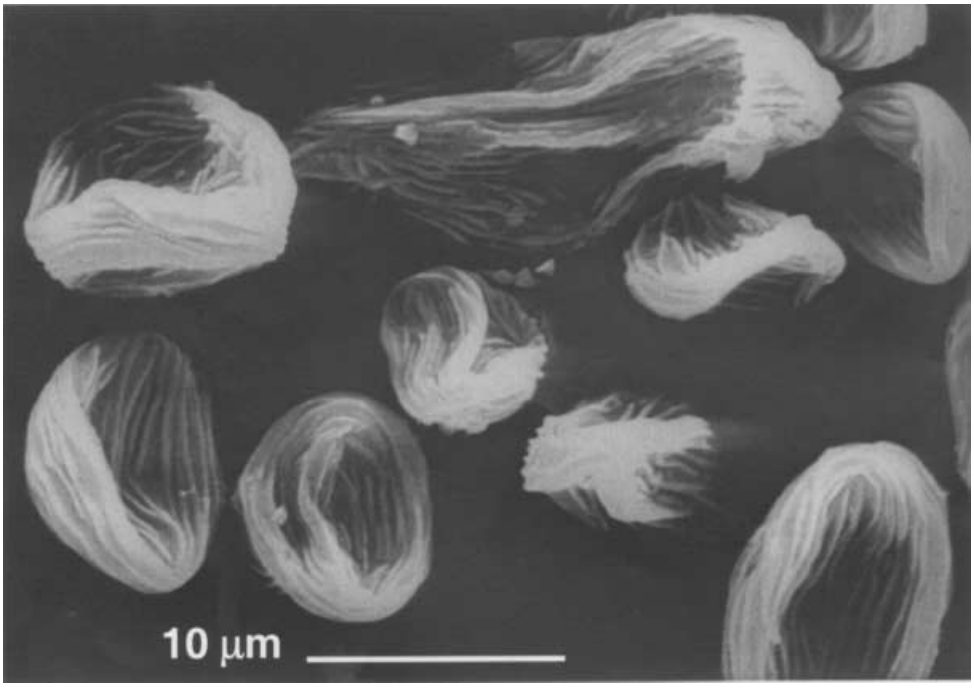

Figure 1. Scanning Electron Micrograph of *Rhizopus* sp. spores taken from corbicular loads of homing *Trigona collina* workers in Deramakot Forest Reserve, Sabah, Malaysia. The material was dried at room temperature

Microscopic analysis showed that the loads were almost pure samples of globular, partly elongate spores that were characterized by a distinctly striate ornamentation and variable size of 7 to 18 microns (Fig. 1). Samples were frozen and transported to the Mycology laboratory at Tübingen. Here the spores were transferred to MA-medium (Gams et al., 1987). Germination was followed by growth of mycelium and finally the production of sporangia. The culture was identified as *Rhizopus* Ehrenberg ex Corda sp. by the shape of its stolones and rhizoids as well as by the characteristic striate ornamentation of the sporangiospores (Zycha et al., 1969). Spore morphology of the culture was identical to that of the spores in corbicular load samples taken from the bees.

Rhizopus sp. are mostly saprophytic molds on a wide range of substrates such as feces and decaying parts of animals and plants (Domsch et al., 1980). Among other fungi they have also been found to spoil provisions in brood cells of soil nesting bees and are suspected to cause some mortality among brood (Linsley and MacSwain, 1952; Batra et al., 1973). This effect, however, is mostly associated with bees ground-nesting in moist substrate, whereas mold-caused mortality of brood is apparently rare in honeybees (Batra et al., 1973). Unfortunately, we have no data on the fate of the mold spores within the colonies of *T. collina*. No detrimental effect was evident, however, and all colonies were alive and populous one and two years after the observations were made. Thus, it is likely that the spores were mixed with pollen reserves within the colony and, at least partly, introduced into the intra-colony flow of liquid food (Sommeijer et al., 1985). Generally, bees possess various potential defenses against microbial infections including cephalic gland secretions, chemicals of nest materials, and possibly colony thermoregulation (Cane et al., 1983; Seeley, 1985).

Several authors have speculated on the benefits of fungal spore collection for bees. Protein content of spores seems low in comparison to pollen (Shaw, 1990), but spores may warrant harvesting simply because they are available in huge quantities (Kempf-Mercado, 1955; Shaw, 1990). Unfortunately, we could not locate the source of the mold spores collected by *T. collina* in our study. However, we speculate that spore collection was indeed related to mass-production of fungal spores triggered by flowering phenology. At the time of our observations the entire study area experienced a heavy dry spell that was accompanied by a regional mass flowering event that involved all strata of the forest and included many canopy trees (Eltz et al., 2001). Thus, mold spore collection took place at a time when large amounts of deceased petals and flower parts were accumulating on the forest floor. Stingless bees can be observed to continue foraging from flowers dropped on the ground (T. Eltz, pers. obs.) and may thus have come in contact with sporangia of *Rhizopus* growing on the decaying flower material. If this scenario is correct, spore-collection may have been favored initially by regular pollen collection from the same site.

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