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Nestmate Recognition of the Stingless Bee *Trigona (Tetragonula) minangkabau* (Apidae: Meliponinae)

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Abstract — Nestmate recognition was studied in the Southeast Asian stingless bee *Trigona (Tetragonula) minangkabau,* a species in which worker oviposition has not been observed in queenright or queenless colonies. When conspecific non-nestmate foragers from queenright and queenless colonies were introduced to the observed colony, they were all rejected by guards. Foragers of a different species *(Trigona (Tetragonisca) angustula)* were also completely rejected. However, conspecific non-nestmate callows were accepted as often as were nestmate callows, although guards recognized the difference. Accepted non-nestmate callows exchanged food with guards equally as much as nestmate callows did.

Many recent studies (reviewed in Michener & Smith 1987; Breed & Bennett 1987; Inoue 1993) have shown that many social insects discriminate nestmates from non-nestmates (Buckle & Greenberg 1981; Breed 1983) and/or conspecifics that are genetically related at different levels (Greenberg 1979; Visscher 1986). The necessity for social insects to be capable of recognizing distinctions such as colony membership (nestmate recognition) and/or kinship (kin recognition) has been assumed to underlie the discriminating behaviors, although theoretical studies (Crozier 1987; Grafen 1990, 1991; Stuart 1991) have shown that it is not easy to separate kin recognition from nestmate recognition, conceptually or experimentally. Studies of nestmate and kin recognition have been made largely on the sweat bee *Lasioglossum zephyrum, Polistes* wasps, the honey bee *Apis mellifera* and ants (Formicidae).

Stingless bees are highly eusocial apid bees (Meliponinae, Apidae: Michener 1974; Sakagami 1982). The number of species is much larger in stingless bees (ca. 400 spp.) compared with honey bees (8 spp.), and stingless bees develop quite complicated brood cell provisioning and oviposition processes (Sakagami 1982; Wittmann et al. 1992). However, kin and nestmate discrimination of stingless bees have scarcely been interpreted from the viewpoint of the recent interest related to the theory of kin selection (Inoue & Roubik 1990 and Breed & Page 1991 are the only studies).

Trigona (Tetragonula) minangkabau is a monogynous Southeast Asian species (Sakagami & Inoue 1985). In this species, worker oviposition has not been observed in either queenright (Sakagami et al. 1983b) or queenless colonies (Tezuka unpub.). Species of this type show the simplest pattern of nestmate discrimination, compared with other stingless bee species in which workers produce males even at queenright colonies (e.g., *Melipona fasciata,* Inoue & Roubik 1990). In the latter, the capability of workers to discriminate nestmates which have different degrees of kinship may increase inclusive fitness, accepting only workers highly related in kinship, and maximizing the contribution of male production of the accepted workers. In the former, on the other hand, such a capability of discrimination does not increase inclusive fitness because nestmates never produce males. Thus, studies of species that do not show worker reproduction should give a simple and basic framework to understand more complicated patterns of recognition, including kin recognition within a colony.

Materials and Methods

Stingless bees

We collected 4 colonies of *Trigona (Tetragonula) minangkabau* (Sakagami & Inoue 1985) in the vicinity of Padang, Sumatra, Indonesia, in March 1988, and imported them to Matsue, Japan, by air in June 1988. The colonies were transferred in flat, wooden observation nests with glass tops. The nests were maintained in the rearing room in the campus of Shimane University in Matsue. Air temperature was maintained between 25°C and 30°C. Nest entrances were connected to a greenhouse in which many flowers were provided. Two queenright nests, A and B, were used for observation, each containing 700-800 workers (about 1/3 of the size of mature colonies, Sakagami et al. 1983a).

There are 5 sequential age classes in task performance of workers (Salmah 1987; Tezuka et al. 1990); callows ($<$ 6 days after emergence), brood carers (6-17), provision handlers (18-30), intranidal guards (31-46) and extranidal guards and foragers ($>$ 46). We can estimate the age class of any given worker by its pigmentation (blackening of the body color, Salmah et al. 1984).

For Experiment 1, we used *Trigona (Tetragonisca) angustula* that was imported from Ribeirão Preto, São Paulo, Brazil. The nests of this species were also maintained in the same way as those of *T. minangkabau.* Workers of *T. angustula* never produce eggs in queenright colonies. The size of the workers is relatively small among stingless bees and is similar to that of *T. minangkabau.*

Experiment 1: Introduction of conspecific and heterospecific foragers

We introduced conspecific non-nestmate foragers from the queenright nest C and from the queenless nest D into the observation nests A and B. Before introduction, we marked the non-nestmate foragers with paint and kept them in a separate box for several hours. For comparison, we also introduced foragers of a different species, *T. angustula.*

We introduced foragers carefully from a hole perforated into the sides of the observation nests (different from the nest entrance) for 6 days (August 2, 3, 14-17, 1988). One or two foragers were introduced at intervals of $>$ 15 min. After introduction, we tried to follow the introduced foragers and recorded responses of guards to them for 0.5-2 h.

Experiment 2: Introduction of conspecific callows

We took out small parts of brood cells that contained ca. 100 pupal cells from nests A and B on July 25 and kept them in sub-nests A' and B'. Adults emerged from those cells were individually marked within a day after emergence and kept in a separate box for several hours. Half of the callows of sub-nest A' were introduced into nest A, and the other half into nest B. The same introduction was done for workers of sub-nest B'.

One to 10 callows were introduced on a daily basis from a side hole of the observation nests for 15 days from July 26 to August 14. Observations lasted for 2-3 h every day (a total of 56 h in each nest) from July 26 to August 21. When we found an introduced bee in the nest during an observation, we tried to record responses of guards to it for up to 5 min.

Results

Experiment 1: Conspecific and heterospecific foragers all rejected

Intranidal guards of nests A and B of *Trigona (Tetragonula) minangkabau* completely rejected the conspecific non-nestmate foragers of the queenright nest C ($n = 6$), and the queenless nest D ($n = 6$), and the *T. (Tetragonisca) angustula* foragers ($n = 2$). All the introduced non-nestmate foragers disappeared from the nests within 1 day after introduction. Responses of guards to these introduced foragers was basically the same among the 3 categories (Fig. 1). Soon after introduction, several guards surrounded the introduced forager and some started biting its legs and wings while the others antennated its body insistently. This antennation continued longer and was more aggressive than the brief antennation of nestmates. The introduced forager then tried to escape from the surrounding guards. Frequently, 1 or 2 guards mounted its body to keep it from escaping. Sometimes the introduced forager got out and guards chased it. When the introduced forager counterattacked a guards, each bit the body of the other. This was the most severe type of fight, "grappling", which resulted in death of both bees.

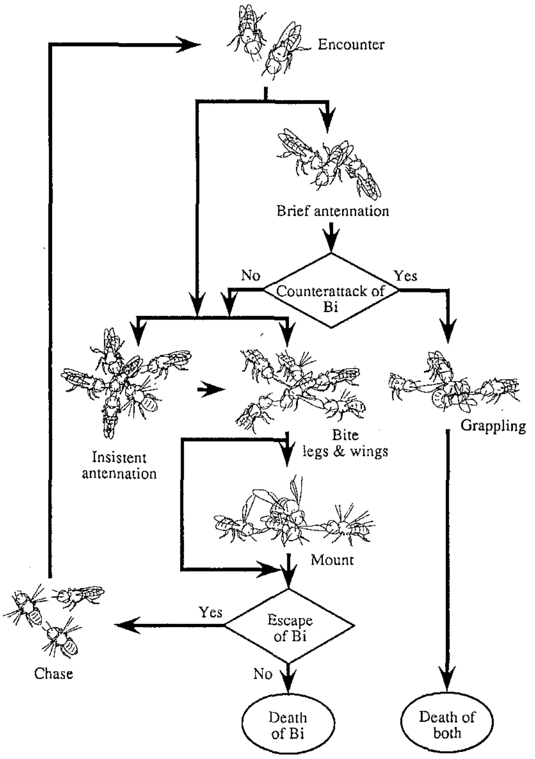

Fig. 1. Behavior sequences of stingless bee guards *Trigona (Tetragonula) minangkabau* after encounter with the introduced bee (Bi) of non-nestmate foragers. When the introduced forager counterattacked a guard, each bit the body of the other, termed "grappling".

Experiment 2: Conspecific callows all accepted

Intranidal guards accepted both non-nestmate and nestmate callows (Fig. 2). Soon after encounter, the intranidal guard briefly inspected the body of the introduced callow with antennae (as they usually did when encountering nestmates). After that, the guard sometimes left and sometimes continued the inspection. In the latter case, the guard often repeated the brief antennal inspection. Occasionally, the guard mounted the callow as an aggressive inspection response. After the inspection, the guard sometimes made trophallaxis with the introduced callow. In all observed cases, the guard finally accepted the introduced callow. The percentages of the introduced callows that survived more than 1 day were 94% (n = 34 in nest A) and 100% (n = 31 in nest B) in nestmates, and 100% (n = 31 in nest A, and n = 33 in nest B) in non-nestmates.

Antennations were frequently observed soon after introduction, and significantly decreased in the following days (Fig. 3). Statistically significantly greater antennation of non-nestmates occurred only 1-4 days after introduction in nest A and 5-20 days in nest B (Fig. 3). The prop-

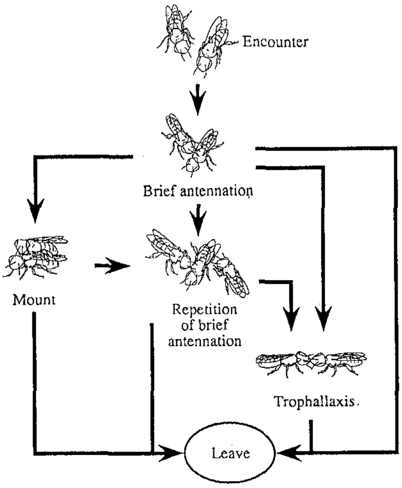

Fig. 2. Behavior sequences of *T. minangkabau* guards after encounter with the introduced workers of nestmate and non-nestamate callows.

ortion of mountings was significantly higher in non-nestmates (Fig. 4). There were no significant differences of the proportion of introduced workers which engaged in trophallaxis (Fig. 5).

Only 6 of the introduced workers were observed to be mounted more than twice after introduction. Four of the introduced workers were observed to mount another worker. For example, B'll (a non-nestmate introduced into nest B) was observed to be mounted 8 times. One of the mountings was by B'24 (another introduced non-nestmate), a females sibling of B'll.

Discussion

Intranidal guards of the stingless bee *Trigona (Tetragonula) minangkabau* properly discriminated workers which could be colony enemies, and they completely rejected conspecific non-nestmate foragers from both queenright and queenless colonies, and *T. (Tetragonisca) angustula* foragers (Exp. 1). This capability to discriminate is necessary, and adequate, for defense of the nest against conspecific and heterospecific robbers under natural conditions, because only foragers can leave nests and potentially function as robbers (by raiding other nests and plundering food supplies, larval provisions and nest construction material).

In contrast to the responses for foragers (Exp. 1), guards accepted non-nestmate callows as well as nestmate callows (Exp. 2). But this does not necessarily mean the absence of nestmate recognition, because they antennated and mounted non-nestmates more frequently than nestmates (Figs. 3 and 4). The guards are regarded to have accepted non-nestmate callows despite recognizing the latters' "source". This would not be harmful for the colony because callows are never robbers.

Although origin of cues for nestmate recognition in *T. minangkabau* may be either genetic or environmental, cue ontogeny would be adaptive enough to discriminate conspecific robbers. The quantitative differences of recognizing behavior of nestmate and non-nestmate callows (Figs. 3 and 4) indicate that recognition cues are derived from pre-emergence factors including genetic bases and/or nest environments such as larval food. The observation that only a

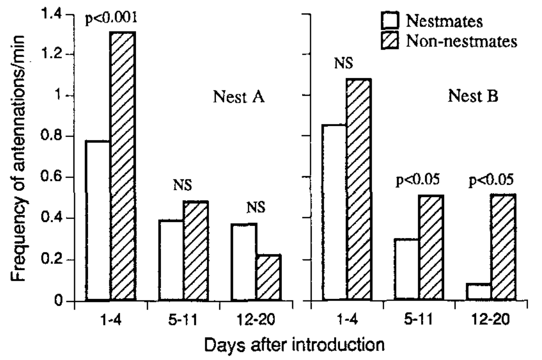

Fig. 3. The frequency of antennations per min directed by *T. minangkabau* guards to introduced callows. (Probabilities between nestmates and non-nestmates were calculated by Mann-Whitney U test.)

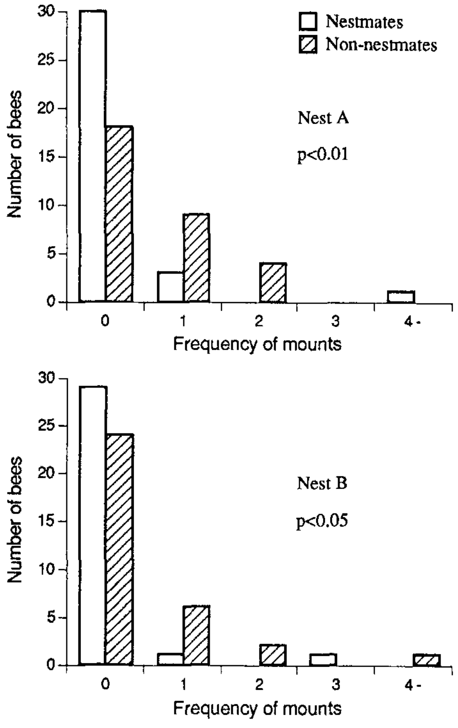

Fig. 4. The frequency distributions of mounts directed by *T. minangkabau* guards to introduced individual callows. (Probabilities between nestmates and non-nestmates were calculated by Mann-Whitney U test.)

few workers introduced as callows were mounted repeatedly (Fig. 4) implies that individual cue differences are not negligible.

The different responses toward non-nestmate foragers and callows (Figs. 1 and 2) indicate that the recognition cues change at least partly after emergence. But frequency of antennations directed toward accepted callows decreased after several days (Fig. 3). This could be because the guards had come to habituate the genetic cues of the introduced callows (which had come from a nest different from the ones that introduced non-nestmate foragers were from), or that the cues of the accepted callows had become mingled with the odor(s) of the introduced nest. An ontogeny of the recognition cues which is determined by both genetic and environmental factors is reported in the honey bee *Apis mellifera* (Breed 1983).

Honey bees have a more elaborate capability of kin and nestmate recognition. This is thought to be adaptive to their social structure, which is relatively complex in comparison with *T. minangkabau.* In the honey bee, kin and nestmate recognition have been studied in some different social contexts, such as agonistic and cooperative behaviors between workers queen rearing, swarming and supersedure (reviewed in Breed & Bennett 1987; see also Page et al. 1989 and Moritz & Hillesheim 1990). The main purposes of those studies are related to the consequences of polyandry (multiple mating of the queen) in the honey bee which produces plural patrilines in a single colony. In fact, the preferential altruism displayed towards full sisters rather than to half sisters within a colony raises the inclusive fitness of the individual worker, and the capability to assess the kinship of nestmates may have evolved from this factor (Carlin & Frumhoff 1990; Page et al. 1990).

Although no evidence has been given for multiple mating in stingless bees (Engels & Imperatriz-Fonseca 1990), there are different contexts in which kin recognition within a colony could be important for species of this subfamily (Meliponinae). In some species of stingless bees, workers produce male eggs even when their mother is still able to lay eggs (Sakagami 1982). In such a situation, workers could favor their own sons, sons that are an extremely important component of worker fitness (Roubik 1990). For example, in *Melipona fasciata,* the percentage of cells which receive male eggs produced by workers is 1%; in *Scaptotrigona barrocoloradensis* this is up to 10-25% (Inoue unpub.). These percentages are much higher than 0.12% reported for the honey bee (Visscher 1989). The greater direct contribution of workers to reproduction of males must affect social structure and the selective value of kin recognition. But in *T. minangkabau,* there is no worker reproduction in queenright or queenless colonies (Sakagami et al. 1983b; Tezuka unpub.). Therefore, in this species, the guards' acceptance of distantly related callows that are experimentally introduced (Fig. 5) does not lower the inclusive fitness of the guards. The variability of guards' discrimination of foragers and callows is consistent with the social structure of this species.

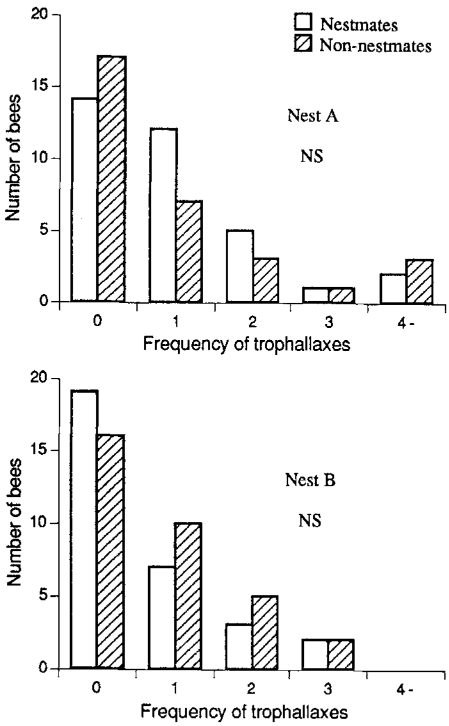

Fig. 5. The frequency distributions of trophallaxes which introduced individual callows of *T. minangkabau* engaged in. (Probabilities between nestmates and non-nestmates were calculated by Mann-Whitney U test.)

As already shown, there is a large variation of contribution of workers to male reproduction among species of stingless bees (Sakagami 1982). Thus, stingless bees are good subjects for studying kin and nestmate recognition. Further comparative studies will reveal that there is true kin recognition (Grafen 1990) within the colony in some species.

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