

## Nestmate discrimination in the African stingless bee *Hypotrigona gribodoi* Magretti (Hymenoptera: Apidae)

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**Abstract** – Sociobiological studies of the highly eusocial stingless bees (Meliponinae) have been almost exclusively restricted to neotropical species. Very little is known about the behaviour of Old World meliponines. Here, we studied nestmate discrimination in the African stingless bee *Hypotrigona gribodoi* Magretti. Interactions between single individuals were observed in arena experiments. It is shown that these bees can discriminate nestmates from non-nestmates. We also studied the behaviour of workers at the nest entrance. Colonies were replaced by other colonies while foragers were outside the nest. Returning foragers entering the entrance tube of the non-nestmates' nest were frequently observed to immediately leave the nest again. Individually marked non-nestmates, on the other hand, which were experimentally introduced directly into the nest, survived and were neither attacked nor driven out of the colony. © Inra/DIB/AGIB/Elsevier, Paris

**stingless bees / Meliponinae / *Hypotrigona* / nestmate discrimination / kin recognition / sociobiology**

### 1. INTRODUCTION

Nestmate recognition and discrimination are of crucial importance for members of colonies of social insects [11, 30]. They have been described in termites [1, 15, 26] as well as in many species of social Hymenoptera such as ants, primitively eusocial wasps, primitively eusocial bees and the highly eusocial honeybee, *Apis mellifera*

[2, 4, 5, 9, 11, 14, 16, 19, 22]. By excluding conspecific non-nestmates, the colonies avoid not only theft of stored food, nesting material and brood or even adult nestmates, but also social parasitism. In honeybees, which can discriminate female [4, 25] and male [17] nestmates from non-nestmates as well as full sisters from half sisters [12, 27], it was shown that both acquired nest odour and genetically determined odour cues serve

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kin and nestmate recognition [7, 8, 13]. The primitively eusocial bee *Lasioglossum zephyrum* discriminates by using individual cues of genetic origin produced by colony members [18].

In meliponine bees (for general meliponine biology see [23, 28, 29]), nestmate recognition has been demonstrated in the most derived neotropical genus, *Melipona*, where encounters between non-nestmates almost always ended with the death of one of the bees [6]. Phylogenetically more ancestral meliponine genera have yet to be studied.

We tested for intraspecific nestmate recognition in *Hypotrigona gribodoi* Magretti (1884), apparently the most common African stingless bee species (for an account on the biology of this species see [3, 10]), which Michener [20] places in one of the least derived positions within the meliponine phylogeny. The sympatric occurrence of the obligate cleptoparasite *Cleptotrigona cubiceps* and the fact that nests of *Hypotrigona gribodoi* are often aggregated in space (Friebe and Kirchner, unpublished) indicate that a well developed system of nestmate recognition and discrimination should be advantageous for this species.

## 2. MATERIALS AND METHODS

The colonies of *Hypotrigona gribodoi* used for the experiments were collected at Lapalala Game Reserve, Northern Province, Republic of South Africa, in June/July 1997. These colonies were situated either in their natural nests inside logs that had been removed from trees, or had been moved to an observation hive (similar to the one described in [24]) where they had re-established themselves. Only fully pigmented workers, which usually are field bees [3], were used for the experiments. They were either caught flying in front of the entrance or taken out of the nests (there was no indication that bees from both groups behaved differently), both by using a small aspirator, and were individually put into clean Eppendorf tubes.

The experimental set-up was similar to the one described for nestmate discrimination exper-

iments with *Melipona* by Breed and Page [6]. In each individual test, three bees from one hive and one from another were separated into two groups of two bees each, one consisting of nestmates and one of non-nestmates, in two arenas made of clean 35-mm Petri dishes. After preliminary recordings of ethograms, the behaviour of running away after antennal contact, subsequently called 'escape response', was chosen as an indicator of discrimination for the experiments. All observations were blind, the bees were unmarked, and the observers were unaware whether the observed bees were nestmates or not until the end of the experiment. Each pair was observed for four periods of 5 min (the pairs were exchanged between the two observers after each 5-min interval). Twenty-one pairs of nestmates and 21 pairs of non-nestmates were formed, i.e. escape responses were recorded for 84 5-min observation periods for nestmates and non-nestmates. Six colonies (C1 to C6) were used as the source of bees for the experiments. Because colonies which are found close to each other in the field might be closely related, we also tested for a correlation between the number of escape responses and the distance between the nest sites of the colonies.

Another series of similar experiments using two colonies was performed on 12 pairs of nestmates and 12 pairs of non-nestmates of which one individual was paint marked, allowing us not only to test for possible effects of paint marks on recognition, but also to test for asymmetries in the interactions indicating possible dominance interactions.

To look at colony or nestmate recognition in a more natural context, some additional observations were made in the field as well as in the lab: colonies in logs (field) or nestboxes (lab) were placed where another nest had been before, either while foragers were outside the nests or when no foragers were outside the nest (because the entrance had been closed early the same morning and was opened just at the beginning of the experiment). Logs very similar to the human eye, with entrance tubes of similar sizes at the same height, and pointing in the same direction were chosen for replacement, the same being true for nestboxes. The behaviour of foragers arriving at these 'foreign' nests at the original location of their own nest was observed.

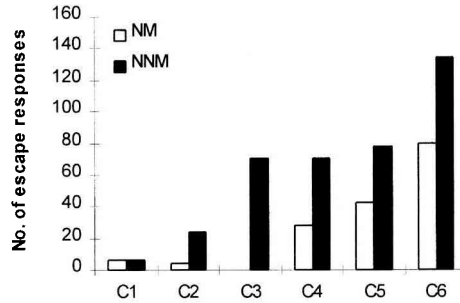
In addition, marked foragers were introduced into other nests. Their behaviour was observed and their time of persistence in the foreign nests was recorded.

### 3. RESULTS

Members of five out of the six colonies showed significantly (G-test,  $P < 0.05$ ) more escape responses when paired with non-nestmates than with nestmates (figure 1). No significant differences between colonies were found, although we saw a weak, non-significant tendency of an increasing number of escape responses with increasing distance from the places where the colonies had been situated in the field. We therefore pooled all data (a total of 543 escape responses) and found significantly more escape responses in pairs of non-nestmates compared to pairs of nestmates (G-test,  $P < 0.001$ ).

The same result was found when interactions between a paint-marked bee and an unmarked bee were observed; the number of escape responses was significantly higher in pairs of non-nestmates compared to pairs of nestmates (G-test,  $P < 0.05$ ). In order to test for possible asymmetries in the interactions, which would indicate that dominance rather than recognition of non-nestmates caused the escape responses, the coefficient of correlation between the escape reactions of both individuals was calculated. This correlation coefficient, which should be negative in the case of dominance interactions, was  $+0.83$  ( $t$ -test,  $P < 0.001$ ) indicating that the interactions were symmetrical.

When colonies were removed and replaced by another while foragers were outside the nest, the bees could be observed approaching the entrance and even climbing into the entrance tunnel. During intervals of 3 min each, the number of entering bees ranged from seven to 11 in four tests, which was not different from the number of bees entering their own nest within 3 min. Almost all of these bees were observed leaving the entrance tube and approaching it again without having flown away further than 10 cm (during intervals of 3 min each, the number of such behaviours was six to 14 in four tests). This behaviour was never



**Figure 1.** Nestmate discrimination in *Hypotrigona gribodoi*. Number of escape responses in arena tests using six colonies. With the exception of C1, significantly more escape responses were observed in pairs of non-nestmates than in pairs of nestmates ( $P < 0.05$ ).

observed during 5 months of field work nor the following 11 months of laboratory experiments for foragers entering their own nest.

When marked non-nestmate bees were artificially introduced into a nest (by briefly opening the covering lid), newly emerged, weakly pigmented bees ( $n = 7$ ) were always accepted and integrated. Foragers ( $n = 9$ ) showed escape responses as described above but were seen for at least 3 d inside the nest, mostly sitting on the brood. Never were they observed performing any tasks inside the new colony. Two individuals that were active foragers at the time of introduction into a foreign nest were still seen inside the foreign nest 46 d after introduction.

### 4. DISCUSSION

Our experiments show that *Hypotrigona gribodoi*, a phylogenetically ancestral stingless bee, like sweat bees, honey bees and the neotropical stingless bee genus *Melipona*, does discriminate between nestmates and non-nestmates. Breed and Page [6] reported that non-nestmate intraspecific encounters of *Melipona scutellaris*, *M. quadrifasciata* and *M. rufiventris* individuals usually ended with the death of one of the animals. In contrast to *Melipona*, biting or even killing of other

individuals was never observed in *Hypotrigena gribodoi* between non-nestmates in our experimental set-up. In *Hypotrigena gribodoi*, the active part in discrimination seems to lie with the intruder rather than the nests' inhabitants. Bees recognizing non-nestmates exhibit escape responses in the arena experiments as well as at the entrance of and inside a non-nestmate nest. Such a mechanism would primarily help foragers to avoid entering and working in a non-kin colony. However, compared to nestmate recognition systems in more derived meliponines and other social insects, discrimination by the intruders should not be evolutionary stable. It should lead to exploitation through the evolution of social parasitism, and finally to the evolution of discrimination mechanisms suitable to defend the colonies against intruders. *Hypotrigena gribodoi* is indeed subject to exploitation. The cleptoparasitic *Cleptotrigona cubiceps*, which according to Michener [20], is not closely related to *Hypotrigena*, frequently raid their nests ([21], and personal observation).

Nestmate discrimination seems thus to be quite different in *Hypotrigena gribodoi* compared to all other systems described for eusocial insects so far. Its non-aggressive mode of action makes it more vulnerable and susceptible to possible intraspecific as well as interspecific exploitation. It would therefore be interesting to comparatively study nestmate recognition in more derived species of stingless bees, which might have evolved a more robust discrimination system, possibly leading to the one described for *Melipona* [6]. Such a comparative approach could possibly reveal a scenario for the evolution of nestmate discrimination in social bees.

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**Résumé – Reconnaissance des congénères de la colonie chez l'abeille sans dard africaine, *Hypotrigena gribodoi* Magretti (Hymenoptera : Apidae).** Il est important, pour les membres des colonies d'insectes sociaux, de pouvoir reconnaître les congénères de la colonie de ceux qui ne le sont pas, puisque les membres des castes stériles peuvent influencer sur leur propre valeur adaptative simplement en coopérant avec des individus apparentés. Des mécanismes de reconnaissance des congénères de la colonie ont été décrits chez les termites, chez les guêpes et les abeilles eusociales primitives et chez l'abeille mellifère, *Apis mellifera*, abeille eusociale évoluée. Chez les Méliponinae, elle n'a été mise en évidence que chez le genre le plus évolué, *Melipona*, où la rencontre entre non congénères de la colonie se termine le plus souvent par la mort de l'un deux.

Nous avons étudié la capacité des ouvrières d'*Hypotrigena gribodoi* à reconnaître les congénères de la colonie. Il s'agit d'une espèce africaine que Michener place à la base de la phylogénie des Meliponinae. Des paires de butineuses provenant de six colonies ont été observées dans une arène constituée par une boîte de Pétri de 35 mm pendant des périodes de 5 min et le nombre de réactions de fuite après contact antennaire a été noté. Chez cinq des six colonies testées, les réactions de fuite étaient significativement plus nombreuses ( $p < 0,05$  au moins) lorsque les abeilles observées n'étaient pas des congénères de la colonie (figure 1). Au total le nombre de réactions de fuite a été très significativement plus élevé ( $p < 0,001$ ) chez les paires d'abeilles constituées de non congénères de la colonie.

On a procédé en outre à des échanges entre colonies du laboratoire et colonies de plein air, de sorte que les butineuses qui rentraient

trouvaient un nid étranger à la place du leur. Elles approchaient du tube d'entrée, pénétraient dedans mais en général en ressortaient pour s'en approcher à nouveau immédiatement.

Des abeilles ont également été marquées et introduites dans un nid étranger. Les ouvrières récemment écloses ( $n = 7$ ) ont été acceptées et intégrées. Les butineuses ( $n = 5$ ) ont présenté un comportement de fuite semblable à celui décrit plus haut, mais elles ont été vues dans le nid durant au moins 3 j, en général sur le couvain. Deux individus ont été vus dans le nid 46 j après leur introduction.

Nos résultats montrent que *Hypotrigona gribodoi* est capable d'une discrimination intraspécifique entre congénères et non congénères de la colonie. La reconnaissance semble être principalement le fait de ces derniers. Ce résultat est discuté dans le cadre de l'évolution. © Inra/DIB/AGIB/Elsevier, Paris

**Meliponinae / *Hypotrigona* / reconnaissance parentèle / discrimination / congénère de la colonie / sociobiologie**

**Zusammenfassung – Intraspezifische Nestgenossinnen-Erkennung bei Arbeiterinnen von *Hypotrigona gribodoi* Magretti (Hymenoptera, Apidae).** Für Mitglieder von Kolonien sozialer Insekten ist es wichtig, zwischen Nestgenossen und Nichtnestgenossen unterscheiden zu können, da vor allem Mitglieder steriler Kasten allein durch Kooperation mit verwandten Individuen Einfluß auf ihre eigene Fitneß nehmen können. Nachgewiesen sind Mechanismen der Nestgenossinnenerkennung u.a. bei Termiten, primitiv eusozialen Wespen und Bienen und der hoch eusozialen Honigbiene, *Apis mellifera*. Unter den Meliponinen wurde Nestgenossinnenerkennung nur für die höchstentwickelte Gattung *Melipona* nachgewiesen, wo ein Zusammentreffen von Nichtnestgenossinnen meist mit dem Tode eines der Kontrahenten endete.

Wir untersuchten Arbeiterinnen von *Hypotrigona gribodoi* Magretti, einer stammesgeschichtlich ursprünglichen afrikanischen Meliponinenart, auf ihre Fähigkeit zur Nestgenossinnenerkennung. Dafür wurden Fura geure aus sechs Kolonien paarweise in einer Arena (35 mm Petrischale) beobachtet und in 5-min Intervallen die Anzahl von Fluchtreaktionen nach Antennenkontakt registriert. Bei 5 der 6 getesteten Kolonien waren diese Fluchtreaktionen signifikant häufiger (mindestens  $P < 0.05$ ), wenn Nichtnestgenossinnen beobachtet wurden als in Tests, in denen sich Nestgenossinnen in der Arena befanden (*Abbildung 1*). Insgesamt wurden hochsignifikant mehr Fluchtreaktionen ( $P < 0.001$ ) in Paarungen von Nichtnestgenossinnen beobachtet.

Ergänzend wurden Kolonien im Feld und im Labor gegeneinander ausgetauscht, so daß heimkehrende Fura geure ein fremdes Nest anstatt des eigenen am entsprechenden Platz vorfanden. Diese flogen den Eingangstabus an und liefen auch hinein, flogen dann aber regelmäßig wieder aus dem Nest aus, um häufig sofort darauf dieses erneut anzufliegen.

Außerdem wurden Bienen markiert und in fremde Nester eingesetzt. Frisch geschlüpfte Arbeiterinnen ( $n = 7$ ) wurden stets akzeptiert und integriert. Fura geure ( $n = 9$ ) zeigten Fluchtverhalten wie oben beschrieben, konnten jedoch mindestens drei Tage lang weiterhin im Nest beobachtet werden, wo sie fast immer auf der Brut saßen. Zwei Tiere wurden noch 46 Tage nach dem Transfer im Nest gesichtet.

Unsere Ergebnisse zeigen, daß *Hypotrigona gribodoi* zur intrasppezifischen Nestgenossinnenerkennung fähig ist. Der Umstand, daß die Erkennung vor allem auf der Seite der Nichtnestgenossinnen stattzufinden scheint, wird in einem evolutionären Kontext diskutiert. © Inra/DIB/AGIB/Elsevier, Paris

**Meliponinae / *Hypotrigona* / stachellose Bienen / Nestgenossinnenerkennung / Verwandtenerkennung / Soziobiologie**

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