Apidologie (1996) 27, 353-359

Original article

Reproductive isolation of *Apis nuluensis* Tingek, Koeniger and Koeniger, 1996 by species-specific mating time

N Koeniger [1], G Koeniger [1], M Gries [1], S Tingek [2], A Kelitu [2]

[1] *Institut für Bienenkunde (Polytechnische Gesellschaft), Fachbereich Biologie der JW Goethe-Universität Frankfurt aM, Karl-von-Frisch-Weg 2, 61440 Oberursel, Germany;*
[2] *Agricultural Research Station, Lagud Seberang PO Box 197, 89908 Tenom, Sabah, Malaysia*

(Received 13 May 1996; accepted 19 August 1996)

Summary — The recent discovery of a morphologically distinct *Apis* type in mountain areas of Sabah raised the question of its taxonomic status. After it became evident that the drone flight period did not coincide with other cavity dwelling *Apis* species of Borneo, a new species, *Apis nuluensis* was described (Tingek et al, 1996). Here drone flight observations and results are reported in detail. A total number of 255 drone flights (50–60 drones) were observed at one colony. The drones started to fly at 1044 hours and the flight activity reached a peak shortly after 1200 hours. The last drones flew at 1312 hours. The complete separation of *A nuluensis* drone flight time from the other two cavity dwelling *Apis* species of Borneo functions as a premating barrier and results in complete reproductive isolation. This seems to be significant because overlap of habitats occurs in the transition zone of the upper dipterocarp forest and lower mountain forest (between 1 500m to 1 700m).

***Apis nuluensis* / reproductive isolation / drone flight / Borneo**

INTRODUCTION

The main component of *Apis* queen's sex attractant seems to be 9-oxo-*trans*-2-decenoic acid (Gary, 1962; Shearer, 1970). Consequently, drones of *Apis mellifera* (Ruttner and Ruttner, 1965), *Apis cerana* (Ruttner, 1973, Punchihewa et al, 1990), *Apis dorsata* (Koeniger et al, 1994) and *Apis koschevnikovi* (unpublished data) are attracted to 9-oxo-*trans*-2-decenoic acid.

Avoidance of interspecific sexual attraction among sympatric *Apis* depends on species specific mating times (Koeniger and Wijayagunesekera, 1976). In Borneo, the daily periods of drone flight are as follows (all hours in local time = GMT + 8): *Apis andreniformis* drones fly from 1200 to 1345 hours, *A cerana* drones start at 1400 hours and cease flying at 1615 hours; *A koschevnikovi* fly between 1645 and 1830 hours, while the drones of *A dorsata*, fly at dusk from 1815 until 1905 hours (Koeniger et al, 1988, 1994).

The recent discovery of a morphologically distinct *Apis* type in mountain areas of Sabah raised the question of whether these bee populations were a distinct race adapted to the specific climatic conditions and vegetation of the highlands. In *Apis* the occurrence of mountain bees is a common phenomenon. In Africa for example, *A mellifera monticola* is restricted to mountain forests on the slopes of the Kilimanjaro while the surrounding habitat of *A mellifera scutellata* is down in the East African Savannah (Meixner et al, 1989). Fertile hybrids between *A m monticola* and European bees are produced. Therefore, the African mountain bee is a race of *A mellifera* (Thrybom and Fries, 1992). Similarly, mountain forms of *A cerana* occur in the Himalayas, which show morphometrically distinct differences (Verma et al, 1994).

A different example is *Apis laboriosa*. Based on morphological differences between *A dorsata* and *A laboriosa* Sakagami et al (1980) concluded that these mountain bees deserved the taxonomic status of a species and, following the original classification of Smith, the name *A laboriosa* was revived.

Ruttner (1988) challenged this decision and argued that the morphological differences were of a quantitative nature and did not by themselves justify assigning a species status. Later, drone flight was observed at one colony and the mating flight period was found to be (in the early afternoon hours) separate from the drone flight period of *A dorsata* (Underwood, 1990). Consequently, the taxonomic status of *A laboriosa* as a species was generally accepted.

In the case of the mountain bees of Sabah, it became evident that the drone flight period did not coincide with other cavity dwelling *Apis* species of Borneo and a new species, *Apis nuluensis,* was described (Tingek et al, 1996). Here the drone flight observations for this new species are reported.

MATERIAL AND METHODS

A colony of *A nuluensis* was found opposite the Gunung Emas Rest House, which is situated at the road which connects Kota Kinabalu and Tambunan (54 km from Kota Kinabalu). The hollow tree in which the colony had built its nest was situated about 150 m above the Rest House on a steep slope at an elevation of 2 040 m. The only entrance of the nest was about 1.6 m above the ground.

For the 4 days of observation, the temperature ranged from 15 °C at 0800 hours to about 18 °C at 1200 hours. In the early afternoon, 20 °C was reached during short periods of sunshine. The sky was cloudy and a light drizzle occurred occasionally in the morning hours between 0800 and 1000 hours.

From a distance of 1 m, we watched the nest entrance for 4 days (23/2/95, 28/2/95, 1/3/95; 2/3/95) from 0800 to 1600 hours (on 23/2/95 from 1100 to 1500 hours only). A further day of observation was done at Tenom (3/3/95) where 12 drones of *A nuluensis* were transported from Gunung Emas in a mixed colony of *A cerana* and *A nuluensis*. We used a timer and departures of drones were counted during periods of 4 min 50 s which were separated by an interval of 10 s, during which we did not watch the entrance, but recorded the observations.

At the end of our observations at Gunung Emas we opened the hollow tree and estimated the colony contained 50–60 drones (many of them flying). We did not find the queen. Part of the *A nuluensis* colony, 12 drones and three combs, were transferred into a hive with queenless *A cerana* workers. The same night it was transported about 200 km and placed in an apiary in ARS Tenom (elevation of Tenom = 300 m). During the next day drone flight was observed. The hive was attacked by Ponerine ants during the following night and the *A nuluensis* drones were killed.

RESULTS

In Gunung Emas we observed a total number of 255 drone flights, which were summarized in 15 min intervals (table I). The first drones started out at 1044 hours and the flight activity reached a plateau after 1115 hours with a peak shortly after 1200

Table I. Number of departing *A nuluensis* drones in intervals of 15 min at the natural nesting site (Gunung Emas).

Interval (hours)	*Date*				Σ	%
	*23/02**	*28/02*	*01/03*	*02/03*		
1045–1100	–	0	0	3	3	1.2
1100–1115	0	0	4	8	12	4.7
1115–1130	0	0	14	13	27	10.6
1130–1145	0	0	12	11	23	9.0
1145–1200	0	3	20	8	31	12.2
1200–1215	9	5	35	12	61	23.9
1215–1230	3	1	22	6	32	12.5
1230–1245	4	6	18	5	33	13.0
1245–1300	1	2	19	6	28	11.0
1300–1315	0	0	4	1	5	2.0

Time of observation from 0800 to 1600 hours (* observed from 1100 to 1500 hours). No drone flights occurred before 1045 and after 1315 hours.

hours. After 1300 hours the flight activity decreased and the last drone started out at 1312 hours. In Tenom the drones started out earlier (1005 hours) and flew even longer (1348 hours) (table II). This was a period of more than 3 h which seemed to be rather long in comparison to the other *Apis* species of Sabah (fig 1).

DISCUSSION

The collection of foraging bees from flowers in the highlands of Sabah during three excursions (years) resulted in ten local samples each containing about 10–30 specimens (Tingek et al, 1996). Though foraging bees were abundant at many places, the thick mountain vegetation and rocky habitats made it impossible to follow homing foragers to their nesting site and we did not succeed in locating a colony. Further, Mount Kinabalu as well as part of the Crocker Range are protected natural reserves with

Table II. Number of departing *A nuluensis* drones in intervals of 15 min in Tenom.

Intervals (hours)	*Departing drones*
1000–1015	2
1015–1030	1
1030–1045	2
1045–1100	2
1100–1115	5
1115-1130	7
1130-1145	5
1145–1200	5
1200–1215	6
1215–1230	4
1230–1245	3
1245–1300	2
1300–1315	1
1315–1330	1
1330–1345	1
1345–1400	1
Σ	48

Date of observation 03/03. Time of observation from 0800 to 1600 hours. No drone flights occurred before 1000 and after 1400 hours.

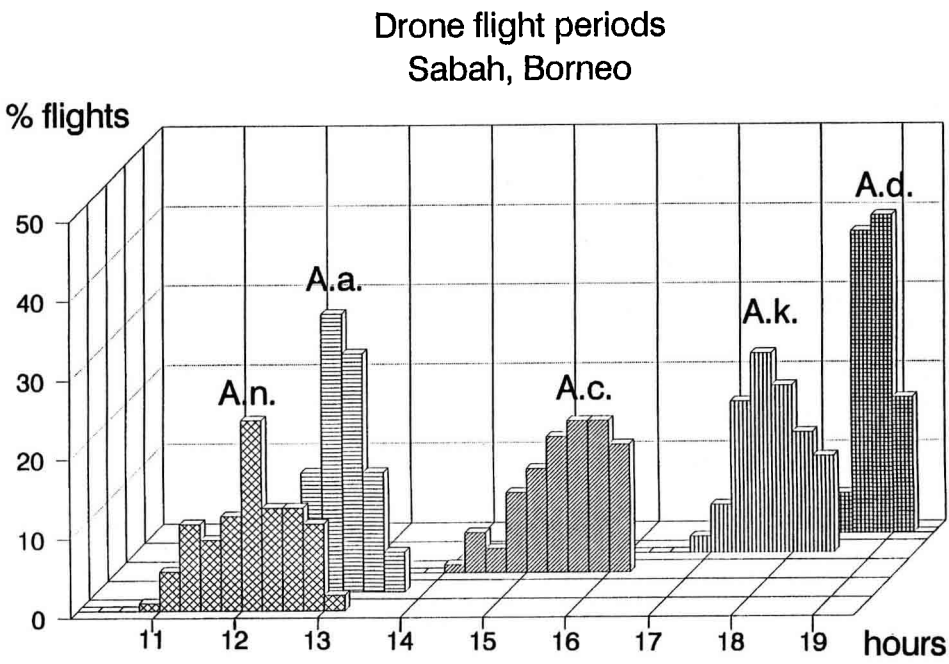

Fig 1. Daily flight activity of drones of five Sabahan *Apis* species. *Aa = A andreniformis; Ac = A cerana; Ad = A dorsata; Ak = A koschevnikovi; An = A andreniformis.*

limited admittance. However, we were informed of a colony near the Gunung Emas Rest House.

The observations on drone flight were made at the single colony of *A nuluensis* which had about 50–60 drones. Later, 12 of these drones were observed in Tenom and flew under very different environmental conditions. The colony was queenless and had about 60% *A cerana* workers. The temperature was 28 °C compared to 18 °C in Gunung Emas. Nevertheless, the drones performed regular flights and the median value of the starts was at 1145 hours which was close to 1200 hours (median in Gunung Emas). Therefore, the flight activity pattern of *A nuluensis* seemed to be rather independent of environmental conditions. Also previous experiments with cross-fostered drones of the closely related species *A cerana* and *A koschevnikovi* indicated that the drone's flight period was fairly independent of colony influence and depended mainly of the genome of the drone (Koeniger et al, 1994). Further, regional variation among colonies of *A cerana* and among *A koschevnikovi* were small and did not exceed 15 min (Koeniger and Wijayagunasekera, 1976; Koeniger et al, 1988; Punchihewa et al, 1990).

The comparison with the other Sabahan *Apis* species (fig 1) shows a slight overlap with the drone flight time of *A andreniformis*. However, the mountainous habitat of *A nuluensis* and the natural distribution of the small free-nesting *A andreniformis* are well separated. According to our collection data, *A andreniformis* is a lowland bee in Sabah and rarely occurs higher than 1 000 m while the lower border of the natural distribution of

A nuluensis is established at an elevation of 1 500 m (upper dipterocarp forest) (Tingek et al, 1996). Thus, interspecific interactions during mating with *A andreniformis* are unlikely.

In the transition zone of the upper dipterocarp forest and lower mountain forest (between 1 500 and 1 700 m), three cavity nesting *Apis* species forage at flowers of the same plant species. There, a complete separation of *A nuluensis* drone flight time from the other two cavity dwelling species of Borneo, *A cerana* and *A koschevnikovi*, functions as a premating barrier (Ruttner, 1988) and results in complete reproductive isolation. In locations from 1 700 to 3 400 m, *Apis nuluensis* is the only honeybee species present, and *A koschevnikovi* and *A cerana* (but not *A nuluensis)* are found below 1 500 m from lowland up to the upper dipterocarp forest (Tingek et al, 1996).

Résumé — Isolement reproductif d'*Apis nuluensis* Tingek, Koeniger et Koeniger, 1996 dû à la période d'accouplement spécifique à l'espèce. Les mâles d'*Apis* sont attirés par l'acide céto-9 décène-2 oïque et l'attraction sexuelle interspécifique entre les espèces sympatriques d'*Apis* est évitée par la présence de périodes d'accouplement spécifiques à chaque espèce. À Bornéo les plages horaires de vol des mâles sont les suivantes : les mâles d'*A andreniformis* volent de 12 h à 13 h 45, ceux d'*A cerana* de 14 h à 16 h 15 ; les mâles d'*A koschevnikovi* volent entre 16 h 45 et 18 h 30 et ceux d'*A dorsata* à la tombée de la nuit de 18 h 15 à 19 h 05. La découverte récente, dans les régions montagneuses de Sabah, d'un type d'*Apis* morphologiquement différent pose la question de son statut taxonomique. La période de vol de ses mâles ne coïncide pas avec celle des autres espèces d'*Apis* nidifiant dans des cavités à Bornéo ; en conséquence une nouvelle espèce a été décrite (Tingek et al, 1996). Ce travail présente les observations faites sur le vol des mâles. À Gunung Emas (altitude 2 040 m) nous avons observé 255 vols de mâles et les avons regroupés par périodes de 15 minutes (tableau I). Les premiers mâles s'envolent à 10 h 44, le dernier à 13 h 12. À Tenom les mâles commencent plus tôt, à 10 h 05, et volent plus longtemps, jusqu'à 13 h 48 (tableau II). Cette période de vol de plus de 3 heures apparaît longue comparée à celles des autres espèces d'*Apis* présentes à Sabah. Les observations sur le vol des mâles ont été faites sur cette seule colonie d'*A nuluensis* qui avait environ 50 à 60 mâles. Par la suite 12 mâles ont été transportés et observés à Tenom, où ils volaient dans des conditions écologiques différentes. Néanmoins les mâles ont effectué des vols réguliers et la moyenne des départs à Tenom s'est située à 11 h 45, contre 12 h à Gunung Emas. Des expériences antérieures avec des transferts réciproques de mâles de deux espèces étroitement apparentées, *A cerana* et *A koschevnikovi*, ont montré que la période de vol des mâles est totalement indépendante de l'influence de la colonie et dépend principalement du génome du mâle (Koeniger et al, 1994). La période de vol des mâles d'*A nuluensis*, totalement séparée de celle des deux autres espèces d'*Apis* nidifiant dans des cavités à Bornéo, fonctionne comme une barrière anti-accouplement et aboutit à un isolement reproductif total. Cela semble important car les habitats se chevauchent. Dans la zone de transition entre le haut de la forêt à diptérocarpacées et le bas de la forêt de montagne (de 1 500 à 1 700 m) les trois espèces d'abeilles butinent les fleurs des mêmes espèces botaniques. Entre 1 700 et 3 400 m, *A nuluensis* est la seule espèce d'abeille mellifère présente. Au dessous de 1 500 m, des plaines jusqu'à la partie supérieure de la forêt à diptérocarpacées, on trouve *Apis koschevnikovi* et *A cerana*, mais pas *A nuluensis*.

***Apis nuluensis* / isolement reproductif / vol de mâles / Bornéo**

Zusammenfassung — Reproduktive Isolation von *Apis nuluensis* Tingek, Koeniger und Koeniger, 1996 durch artspezifische Paarungszeiten. Die 9-Oxo-trans-2-Decensäure ist für die Drohnen aller *Apis*-Arten attraktiv und die Vermeidung interspezifischer sexueller Anziehung zwischen sympatrischen *Apis* - Arten beruht auf artspezifischen Paarungszeiten. Die täglichen Drohnenflugzeiten in Borneo verteilen sich folgendermassen : *Apis andreniformis*-Drohnen fliegen zwischen 12.00 h und 13.45 h, *Apis cerana*-Drohnen starten um 14.00 h und stellen ihren Flug um 16.15 h ein; *Apis koschevnikovi*-Drohnen fliegen zwischen 16.45 und 18.30 h, während Drohnen von *Apis dorsata* in der Dämmerung von 18.15 bis 19.05 h ausfliegen. Nach der kürzlichen Entdeckung eines morphologisch unterschiedlichen *Apis* - Typus erhob sich die Frage nach dessen taxonomischen Status. Nachdem offensichtlich geworden war, daß deren Drohnenflugzeit nicht mit der der anderen höhlenbewohnenden *Apis*-Arten von Borneo übereinstimmte, wurde sie als eine neue Art *Apis nuluensis* beschrieben (Tingek et al 1996). Hier geben wir eine genaue Beschreibung der Drohnenflugzeiten. In Gunung Emas (Höhe über dem Meeresspiegel 2040 m) beobachteten wir insgesamt 255 Drohnenflüge, die in Intervallen von 15 min zusammengefaßt wurden (Tabelle I). Die ersten Drohnen starteten um 10.44 h und der letzte Drohn um 13.12 h. In Tenom starteten die Drohnen früher, um 10.05 h und flogen länger, bis 13.48 h (Tabelle II). Dieser Zeitraum von mehr als drei Stunden war im Vergleich zu den anderen *Apis*-Arten von Sabah relativ lang (Abb 1). Die Beobachtungen der Drohnenflugzeiten wurden an einem Volk durchgeführt, das etwa 50 bis 60 Drohnen enthielt. Später wurden 12 nach Tenom überführte Drohnen beobachtet, wo sie unter unterschiedlichen Umgebungsbedingungen ausflogen. Dort führten sie nichtsdestoweniger regelmäßige Paarungsflüge aus mit einem Median bei 11.45 h in Tenom im Vergleich zu 12.00 h in Gunung Emas. Frühere Experimente mit in Völkern der gegenseitigen Arten aufgezogenen Drohnen der nahverwandten Arten *Apis cerana* und *A koschevnikovi* hatten darauf hingewiesen, dass die Drohnenflugzeiten ziemlich unabhängig vom Einfluß der Völker und im wesentlichen vom Genom der Drohnen bestimmt sind. Die völlige zeitliche Trennung der Drohnenflugzeiten von *Apis nuluensis* von den anderen beiden höhlenbewohnenden *Apis*-Arten hat die Funktion einer Paarungssperre mit dem Ergebnis einer völligen reproduktiven Isolation. Dies ist bedeutsam, da die Habitate sich überschneiden. In der Übergangszone zwischen dem oberen dipterocarpen Wald und dem unteren Bergwald (zwischen 1500 m und 1700 m) besammeln alle drei Arten Blüten der gleichen Pflanzenarten. Oberhalb von 1700 m und bis zu 3400 m ist *Apis nuluensis* die einzige Honigbienenart. Unterhalb 1500 m, vom Unterland bis zu dem oberen dipterocarpen Wald werden *Apis koschevnikovi* und *Apis cerana*, jedoch nicht *Apis nuluensis* gefunden.

***Apis nuluensis* / reproduktive Isolation / Drohnenflug / Borneo**

REFERENCES

Gary NE (1962) Chemical mating attractants in the queen honeybee. *Science* 136, 773-774

Koeniger G, Koeniger N, Tingek S (1994) Crossfostered drones of *Apis cerana* (Fabricius 1793) and *Apis koschevnikovi* (Buttel-Reepen 1906) fly at their species specific mating times. *Insectes Soc* 41, 73-78

Koeniger N, Wijayagunasekera HNP (1976) Time of drone flight in the three Asiatic honeybee species (*Apis cerana*, *Apis florea*, *Apis dorsata*). *J Apic Res* 15, 67-71

Koeniger N, Koeniger G, Tingek S, Mardan M, Rinderer TE (1988) Reproductive isolation by different time of drone flight between *Apis cerana* Fabricius 1793 and *Apis vechti* (Maa, 1953). *Apidologie* 19, 103-106

Koeniger N, Koeniger G, Tingek S, Kalitu A, Mardan M (1994) Drones of *Apis dorsata* (Fabricius 1793) con-

gregate under the canopy of tall emergent trees in Borneo. *Apidologie* 25, 249-264

Meixner M, Ruttner F, Koeniger N, Koeniger G (1989) The mountain bees of the Kilimanjaro region and their relation to neighbouring bee populations. *Apidologie* 20, 165-174

Punchihewa RWK, Koeniger N, Koeniger G (1990) Congregation of *Apis cerana indica* drones in the canopy of trees in Sri Lanka. *Apidologie* 16, 201-208

Ruttner F (1973) Drohnen von *Apis cerana* auf einem Drohnensammelplatz. *Apidologie* 4, 41-44

Ruttner, F (1988) *Biogeography and Taxonomy of Honeybees*. Springer Verlag, Berlin, 284

Ruttner F, Ruttner H (1965) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. II. Beobachtungen an Drohnensammelplätzen. *Z Bienenforsch* 8, 1-9

Sakagami SF, Matsumura T, Ito K (1980) *Apis laboriosa* in Himalaya, the little known world's largest honey bee (Hymenoptera, Apidae). *Insecta Matsumurana* 19, 47-77

Shearer DA, Boch R, Morse RA, Laigo FM (1970) Occurrence of 9-oxodec-*trans*-2-enoic acid in queens of *Apis dorsata*, *Apis cerana* and *Apis mellifera*. *J Insect Physiol* 16, 1437-1441

Thrybom B, Fries I (1992) Development of infestations by *Varroa jacobsoni* in hybrid colonies of *Apis mellifera monticola* and *Apis mellifera ligustica*. *J Apic Res* 30, 151-155

Tingek S, Koeniger G, Koeniger N (1996) Description of a new cavity nesting species of *Apis* (*Apis nuluensis*) from Sabah, Borneo with notes on its occurrence and reproductive biology (Hymenoptera: Apoidea: Apini). *Senckenbergiana Biol* 76, 115-119

Underwood, BA (1990) Time of drone flight of *Apis laboriosa* Smith 1871 in Nepal. *Apidologie* 21, 501-504

Verma LR, Mattu VK, Daly HV (1994) Multivariate morphometrics of the Indian honeybee in the Northwest Himalayan region. *Apidologie* 25, 203-223