Apidologie 30 (1999) 339–346

Original article

Observations on multiple mating flights of *Apis dorsata* queens

Nguyen Quang Tan[a, b]*, Makhdzir Mardan[a], Pham Hong Thai[b], Phung Huu Chinh[c]

[a] Bee Net Asia, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, DE, Malaysia
[b] Bee Research Unit, University of Agriculture & Forestry, Thu Duc, Ho Chi Minh City, Vietnam
[c] Bee Research Center, Lang Ha, Dong Da, Ha Noi, Vietnam

(Received 18 April 1997; revised 15 November 1998; accepted 10 January 1999)

Abstract – This observation is aimed at providing information for a reasonable comparative study on reproductive biology among the honeybee species. The research was carried out in 1996 in the submerged *Melaleuca* forest of southern Vietnam, where low-nesting colonies on man-made supports, rafters, allowed us to make detailed observations on the queens. Flights of six newly emerged queens were observed and after their final mating flights, queens were dissected to count the sperm number. The five investigated queens took their first flights 6 ± 1 (mean ± SD) days after emergence. Four queens took orientation flights of less than 3 min. One queen flew to mate without any orientation flight. Mating flights happened around sunset and lasted 15.4 ± 4.3 (n = 14) min. A queen undertook two to four mating flights and after fully mating, she had 5.5 ± 0.9 (n = 5) million sperm in her spermatheca. This study indicated the extreme polyandry in *A. dorsata*. © Inra/DIB/AGIB/Elsevier, Paris

***Apis dorsata* / queen flight / Vietnam / sperm number**

1. INTRODUCTION

Time of drone mating flights has been studied in *Apis mellifera* by Taber [25] and Ruttner [22], in *A. cerana* [8, 9, 20, 33], *A. florea* [8, 20], *A. andreniformis* [20], *A. koschevnikovi* [9, 12], *A. laboriosa* [30] and *A. dorsata* [8, 13, 20, 26, 28]. Such observations have also been made on queens of *A. mellifera* by Roberts [21], Alber et al. [1] and Woyke [32], on queens of *A. cerana* [23, 33], *A. florea* [10] and *A. koschevnikovi* [12]. However, very little is known about mating flights of *A. dorsata* and

* Correspondence and reprints
E-mail: nqtan@bdvn.vnd.net

A. laboriosa queens. Therefore, studies on mating flights of *A. dorsata* and *A. laboriosa* queens are needed to provide a reasonable comparison of reproductive biology among honeybee species.

In this study, we determined the day of the first flight of virgin queens, the time and duration of a queen flight and number of sperm in spermatheca of newly mated queens of *A. dorsata*.

2. MATERIALS AND METHODS

This research was carried out in the submerged *Melaleuca* forest of U Minh (longitude 104°59' E and latitude 9°27' N), Ca Mau Province, southern Vietnam, from April to September 1996. Here, there are many low-nesting colonies on 'rafters', which are man-made supports to lure *A. dorsata* colonies to nest [2, 14, 16, 27, 29]. These low-nesting colonies permitted observations on the mating flights of the queens. The colonies were only about 1.0–1.5 m above ground level and the rafters with the colonies could be moved from the forest to the housing area for observation.

Queen cells were obtained from natural or dequeened colonies. Some queen cells containing mature pupae were cut out from the comb together with some surrounding cells. Each was kept in its natural orientation in a small plastic cage and placed into an incubator (35 °C and 60 % relative humidity). The emergence of virgin queens was checked every 6 h. The newly emerged virgin queen was marked with a numbered plastic disc on her thorax and introduced into a nucleus colony. Each nucleus colony was small (about 30 cm × 20 cm) to facilitate easy observation on the queen, but had sufficient food and young bees to take care of the queen.

Every day we observed the virgins from 18.00 to 19.00 hours and recorded the time of their mating flights. The local time in Vietnam is GMT + 7.

2.1. Nucleus colony

We smoked a colony of about 50 cm × 30 cm in size. Most of the elder bees and the queen flew out. We moved the comb with young bees from forest to the backyard of our house. Outer parts of the comb (which usually contained young brood) were cut off, so that the remaining comb (which usually contained sealed and large larva brood) could be covered with the bees. The size of the comb was about 30 cm × 20 cm after cutting.

2.2. Sperm count

Queens were dissected for collections of spermathecae and sperm were counted according to Koeniger et al. [10, 11]. Each spermatheca was placed in 1 mL of physiological saline (0.9 % NaCl) and ruptured with fine forceps. Then, 4 mL of distilled water were added. The emerging spermatozoa were dispersed evenly in the fluid by heavily blowing air through it with a syringe. Sperm were then counted using a haemocytometer (improved Neubauer).

3. RESULTS AND DISCUSSION

The five observed queens usually gave very clear signals before they flew to mate. They rushed over the comb from one side to the other, then ran over the workers and took off for flight. The age at which the queens took flights and the duration of their flights are summarized in *table I*. The time of queen flights are shown in *table II* along with the time of drone flights 6 days after emergence in the same area.

On average, virgin queens of *Apis dorsata* took their first flights 6 ± 1 days after emergence. This is within the range of mating flights for the other honey bee species.

Table I shows that flight duration was bimodal. Queens took flights of either less than 3 min or longer than 9 min; no flights between 3 and 9 min were observed in 22 flights witnessed. Therefore, we presumed that the flights of less than 3 min were orientation flights, and those longer than 9 min were mating flights. Queen 1 did not take any orientation flights prior to her mating flights but all other queens did. Queens 2, 3, 4 and 5 made orientation flights before they flew to mate 1, 4, 1 and 3 days later, respectively. Orientation flights were as short as 21 s and as long as 3 min.

Table I. Ages of the queens (days) and durations (min/s) of their flights.

Queen	1st flight	2nd flight	3rd flight	4th flight	5th flight	6th flight	Remark/ Sperm number	Duration (min) of mating flights: mean ± SD (*n*)
1	7 d 13 min	8 d 22 min	9 d 18 min	11 d 15 min	no flight	no flight	14 d abscond	17.0 ± 3.9 (4)
2	**5 d** **21 s**	6 d 13 min	7 d 12 min	8 d 16 min	9 d 19 min	no flight	11 d 5.4 million	15.0 ± 3.2 (4)
3	**5 d** **24 s**	**7 d** **1 m**	**8 d** **1 min**	9 d 12 min	10 d 17 min	11 d 20 min	13 d 7.0 mio	16.3 ± 4.0 (3)
4	**7 d** **2 min**	**8d** **3 min**	8 d 9 min	not observed	not observed	no flight	14 d 5.3 mio	9.0
5	**6d** **30 s**	**6d** **2 min**	9 d 21 min	10 d 9 min	no flight	no flight	12 d 4.6 mio	15.0 (2)
6	not observed	not observed	not observed	not observed	not observed	not observed	42 d 5.2 mio	
Mean ± SD (*n*)	6 d ± 1 (5)						5.5 ± 0.9 (5)	15.4 ± 4.3 (14)

Flights in shaded cells are presumed to be orientation flights (less than 3 min).

Table II. Local time (GMT +7) of mating flights of *A. dorsata* drones and queens in *Melaleuca* forest, Vietnam (longitude 104°59' E and latitude 9°27' N).

	Start	Finish	Remark
Drone flights (Tan et al. [28, 29])			
Colony 1			
27 April 1996	18.14	18.40	
28	18.14	18.38	
29	18.15	18.39	
30	18.08	18.36	cloudy sky
1 May	18.09	18.36	
Colony 2			
28 April 1996	18.14	18.39	
29	18.15	18.38	
30	18.12	18.35	cloudy sky
1 May	18.11	18.36	
2	18.12	18.36	
Queen flights			
Queen 1			
15 May 1996	18.27	18.40	
16	18.24	18.46	
17	18.24	18.42	
19	18.24	18.39	
Queen 2			
22 May 1996	18.28	18.41	
23	18.25	18.37	
24	18.25	18.41	
25	18.25	18.44	
Queen 3			
5 June 1996	18.37	18.49	
6	18.33	18.50	
7	18.31	18.51	
Queen 4	1	1	
5 June 1996	18.40	18.49	
Queen 5			
13 June 1996	18.27	18.48	
14	18.29	18.38	

According to Gary [4] some virgin queens of *A. mellifera* took short orientation flights before flying to mate, but the other queens flew out for mating without an orientation flight. This is the same in our study on the five virgin queens of *A. dorsata*.

Earlier local times of drone flights in *A. dorsata* have been reported in some areas in Asia. They are from 18.00 to 18.45 hours in Sri Lanka [8], 18.15 to 18.45 hours in southeastern Thailand [20], and about 18.27 to 18.52 hours in Borneo, Malaysia [13]. Our earlier observation on drone flights in the same area showed that *A. dorsata* drones flew from 18.08 to 18.40 hours [26, 28]. In comparison to the queen, drone mating flight times are coincidental (*table II*). *A. dorsata* queens and drones fly to mate around sunset.

Roberts [21], Alber et al. [1] and Woyke [32] reported that the duration of a queen mating flight in *A. mellifera* was 15.8, 18.0 and 21.0 min, respectively. For *A. cerana*, the duration has been reported to be 30.8 min, ranging from 17 to 46 [23] and 27 min, ranging from 20 to 39 [33]. In *A. florea*, it was from 18 to 30 min [10] and in *A. koschevnikovi*, it averaged 19 min [12]. The duration of a *A. dorsata* queen mating flight in this study was 15.4 ± 4.3 min (n = 14), somewhat shorter in comparison with other honeybee species.

Results from *table I* shows that an *Apis dorsata* queen flew two to four times to mate (queen 5 made only two mating flights, queen 3 made three flights, and queens 1 and 2 made four). Ruttner et al. [23, 24] and Woyke [33] reported that repeated matings may occur in *A. mellifera* and *A. cerana*. Our study with *A. dorsata* shows that repeated mating flights are also usual in this species. Four queens of *A. dorsata* made from two to four mating flights. The number of mating flights taken by an *A. dorsata* queen is higher than those reported in other honey bee species. This is, perhaps, a distinction of *A. dorsata* and it also supports the high number of matings estimated in *A. dorsata* queens (16–27) [15, 18].

The queen that mates with many drones can produce a genetically diverse worker population, which may give the colony better chances of adapting to the environment. Nesting in the open air and often migrating in the tropics and subtropics of Asia, *A. dorsata* colonies face many predators and challenges of environment. It is possible that the number of matings in *A. dorsata* is higher than in other species because the genetically more diverse the colony, the better may be its flexibility in adapting to the environment.

Our results show that the average number of sperm in spermatheca of a newly mated queen of *A. dorsata* was 5.5 ± 0.9 (n = 5) million (very few sperm were found in oviducts). The lowest number was 4.6 and the highest was as many as 7.0 million (*table I*). These numbers are much higher than those reported by Koeniger et al. [11], Woyke [34] and Tan et al. [28] (*table III*). The difference is possibly because our estimates were made from newly and fully mated queens while the earlier results were from queens of unknown age. It is clear that queens use sperm during their life; so older queens contain correspondingly less sperm. Harbo and Szabo [5] reported that after a year the queen of *A. mellifera* lost 1.5 million sperm. In comparison with *A. mellifera*, size of spermatheca and number of sperm in an *A. dorsata* queen are the same. Data on other *Apis* species are shown in *table III*.

Table III. Diameter (mm) and sperm number (million) of spermatheca in some honeybee species.

Species	Size	Authors	Sperm number	Authors
A. dorsata	1.10	Koeniger et al. [11]	3.7	Koeniger et al. [11]
	1.15	Woyke [34]	2.6	Woyke [34]
			2.4	Tan et al. [28], Tan [29]
			5.5	this study
A. mellifera	1.14	after Koeniger et al. [11]	4.7	after Koeniger et al. [11]
			5.5	Harbo and Szabo [5]
A. cerana	0.98	Woyke [33]	1.3	Woyke [33]
A. koschevnikovi			2.0–3.0	Koeniger et al. [12]
A. florea	0.78	Koeniger et al. [10]	1.2	Koeniger et al. [10]
A. andreniformis	0.80	Koeniger et al. [10]	1.0	Koeniger et al. [11]

In *Apis*, drones are monogamous because they die after mating; and the queens are polyandrous. By counting sperm in seminal vesicles of drones and oviducts/spermathecae of queens, numbers of drones mating with a queen were estimated. They were seven to ten in *A. mellifera* [4, 7], ten in *A. cerana* [33], two to three in *A. florea* [10], seven to eight in *A. andreniformis* [11], seven to twelve in *A. koschevnikovi* [12] and at least two in *A. dorsata* [11]. According to our earlier study in the same area [26, 28], the number of sperm in a sexually mature drone was 1.24 ± 0.39 ($n = 31$) million, which showed that the number of matings was five in *A. dorsata*. However, does a drone ejaculate all or only part of its sperm during copulation? It is unknown whether the queens of *A. florea*, *A. andreniformis* and *A. dorsata* receive all or part of the sperm ejaculated from drones. Therefore, the above method can only give the minimum numbers of mating.

Recently, by the DNA microsatellite variability technique, the numbers of matings were reported to be 13.8 in *A. mellifera* [3], 8.0 in *A. florea* [17], 18.0 in *A. dorsata* [15], 26.7 in *A. dorsata* [18] and 13.5 in *A. andreniformis* [19].

In *A. mellifera* and *A. cerana*, a mating sign is thought to facilitate the identification of the queen by the following drones and thus reduces the mating flight time [6, 7]. However, in *A. florea* there is no mating sign found on the queens after mating [10].

In our study, we did not see mating signs on the five queens during their 14 mating flights. However, Woyciechowski et al. [31] found 16 queens with and 12 queens without mating signs in their experiment with *A. mellifera*. Therefore, the answer for *A. dorsata* should be reserved until a larger number of queens are investigated. Moreover, the mating signs of *A. dorsata* may be too small to see without catching the returning queens for closer examination.

According to Koeniger [7], there are mainly two mechanisms of sperm transfer from drones to a queen. The first, as in *A. mellifera* and *A. cerana* (and recently in *A. koschevnikovi* [12]), is that sperm is transferred from drones to oviducts of the queen and after that only 5–10 % of them reach the spermatheca. The second, as in *A. florea* (and *A. andreniformis*, perhaps), is that drones transfer sperm directly into the spermatheca. Further study on sperm transfer mechanism in *A. dorsata* is strongly suggested.

ACKNOWLEDGEMENT

This research was granted by BEENET ASIA/IDRC for the Masters Study of Mr Nguyen Quang Tan. We would like to thank Professor Nikolaus Koeniger and Dr Gudrun Koeniger for their comments on the paper.

Résumé – Observations sur les vols multiples de fécondation chez les reines d'*Apis dorsata* Fabr. Les vols de fécondation ont été étudiés chez les reines d'*Apis mellifera*, *A. cerana*, *A. florea*, *A. koschevnikovi* et *A. andreniformis*, mais on sait peu de choses en ce qui concerne les reines d'*A. dorsata*. Cette étude visait à observer le comportement d'accouplement des reines d'*A. dorsata* pour obtenir des données permettant une étude comparative de la biologie de la reproduction chez les différentes espèces d'*Apis*. Le recherche a été faite en 1996 dans la forêt submergée de *Malaleuca* du sud du Vietnam. Là, de nombreuses colonies nidifient à faible hauteur sur des supports en bois suspendus par les apiculteurs pour attirer les colonies, ce qui rend possible des observations détaillées des vols de fécondation des reines. Habituellement, de telles observations sont impossibles car cette espèce a tendance à nidifier dans des endroits élevés. Six reines récemment écloses ont été marquées et introduites dans des nuclei. Nous avons observé les reines de chaque colonie de 18 à 19 h et noté leur

vols. Les reines ont été disséquées après leur dernier vol nuptial et les spermatozoïdes présents dans la spermathèque ont été dénombrés.

Les cinq reines observées ont accompli leur premier vol 6 ± 1 j (moyenne ± écart type) après leur émergence. Quatre reines ont fait des vols d'orientation inférieurs à 3 min et une reine s'est envolée pour s'accoupler sans avoir fait de vol d'orientation (*tableau I*). Les vols de fécondation ont eu lieu au moment du coucher du soleil (*tableau II*) et ont duré 15,4 ± 4,3 min ($n = 14$). Les reines ont fait entre deux et quatre vols de fécondation et possédaient en moyenne 5,5 ± 0,9 ($n = 5$) millions de spermatozoïdes dans leur spermathèque après le dernier vol de fécondation (*tableau I*). Cette étude confirme, d'une part, la forte polyandrie déjà mentionnée chez *A. dorsata* dans de précédents rapports et, d'autre part, les hypothèses portant sur l'évolution de la forte polyandrie chez le genre *Apis*.

***Apis dorsata* / vol de fécondation / Vietnam / nombre de spermatozoïdes**

Zusammenfassung – Beobachtungen der mehrfachen Paarungsflüge von *Apis dorsata* Königinnen. Über Paarungsflüge von *Apis mellifera* , *Apis cerana* , *Apis florea* , *Apis koschevnikovi* und *Apis andreniformis* liegen Untersuchungen vor, aber über Flüge von *Apis dorsata* Königinnen ist nichts bekannt. Diese Studie über das Paarungsverhalten der *Apis dorsata* Königinnen soll Daten für eine schlüssige vergleichende Betrachtung des Paarungsverhaltens der *Apis* Arten liefern.

Die Forschungen wurden 1996 in den Melalucawäldern der Überschwemmungszone in Südvietnam durchgeführt. Dort nisten viele *Apis dorsata* Völker in geringer Höhe an 'Raftern', das sind spezielle, von Imkern aufgehängte Balken zur Anlockung der Völker. Diese Bedingungen ermöglichten uns detaillierte Beobachtungen über die Hochzeitsflüge, die an den meisten anderen Nistplätzen nicht möglich sind, da die Waben normalerweise in großer Höhe gebaut werden.

Sechs frisch geschlüpfte Königinnen wurden markiert und in Kleinvölker eingeweiselt. Bei jedem Volk beobachteten wir die Königinnen von 18 bis 19.00 Uhr und protokollierten die Flüge. Nach ihrem letzten Flug wurden die Königinnen seziert und die Anzahl der Spermatozoen in der Spermatheka bestimmt.

Bei 5 untersuchten Königinnen fand der erste Flug 6,0 ± 1,0 Tage nach dem Schlupf statt. Vier Königinnen machten einen Orientierungsflug von weniger als 3 Minuten. Eine Königin flog ohne vorherigen Orientierungsflug. Die Paarungsflüge fanden bei Sonnenuntergang statt und dauerten 15,4 ± 4,3 min.

Im Durchschnitt flogen Königinnen 2–4 mal zur Paarung aus, nach voller Paarung hatten sie 5,5 ± 0,9 Millionen Spermatozoen in der Spermatheka. Diese Beobachtung stützt vorherige Berichte über eine extreme Polyandrie bei *Apis dorsata* und die Hypothesen der Evolution der extremen Polyandrie in der Gattung *Apis*.

***Apis dorsata* / Paarungsflüge der Königinnen / Vietnam /Anzahl der Spermatozoen**

REFERENCES

[1] Alber M., Jordan R., Ruttner F., Ruttner H., Von der Paarung der Honigbiene, Z. Bienenforsch. 3 (1955) 1–28.

[2] Crane E., Luyen V.V., Mulder V., Ta T.C., Traditional management system for *Apis dorsata* in submerged forests in southern Vietnam and central Kalimantan, Bee World 74 (1) (1993) 27–40.

[3] Estoup A, Solignac M., Cornuet J.-M., Precise assessment of the number of patrilines and of genetic relatedness in honey bee colonies, Proc. R. Soc. Lond. B 258 (1994) 1–7.

[4] Gary N.E., Activities and behaviour of honey bees, in: The Hive and the Honey Bee, Dadant & Sons, Hamilton, Illinois, 1975, pp. 185–264.

[5] Harbo J.R., Szabo T.I., A comparison of instrumentally inseminated and naturally mated queens, J. Apic. Res. 23 (1) (1984) 31–36.

[6] Koeniger G., Mating sign and multiple mating in the honeybee, Bee World 67 (4) (1986) 141–150.

[7] Koeniger G., Diversity in *Apis* mating system, in: Smith D.R. (Ed.), Diversity in the Genus *Apis*, Westview Press, Oxford and IBH Publishing Co. Pvt. Ltd., 1991, pp. 119–212.

[8] Koeniger N., Wijayagunasekera H.N.P., Time of drone flight in the three Asiatic honeybee species, J. Apic. Res. 15 (2) (1976) 67–71.

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

[10] Koeniger N., Koeniger G., Wongsiri S., Mating and sperm transfer in *Apis florea*, Apidologie 20 (1989) 413–418.

[11] Koeniger G., Koeniger N., Mardan M., Punchihewa R.W.K., Otis G., Numbers of spermatozoa in queens and drones indicate multiple mating of queens in *Apis andreniformis* and *Apis dorsata*, Apidologie 21 (1990) 282–286.

[12] Koeniger N., Koeniger G., Tingek S., Mating flights, number of spermatozoa, sperm transfer and degree of polyandry in *Apis koschevnikovi* (Buttel-Reepen, 1906), Apidologie 25 (1994) 224–238.

[13] Koeniger N., Koeniger G., Tingek S., Kalitu A., Mardan M., Drones of *Apis dorsata* (Fabricius 1793) congregate under the canopy of tall emergent trees in Borneo, Apidologie 25 (1994) 249–264.

[14] Mardan M., Rafter beekeeping with the Asiatic giant honeybee (*Apis dorsata*) in Vietnam, Beenet Online 1 (1) (1993)12–13.

[15] Moritz R.F.A., Kryger P., Koeniger N., Estoup A., Tingek S., High degree of polyandry in *Apis dorsata* queens detected by DNA microsatellite variability, Behav. Ecol. Sociobiol. 37 (1995) 357–363.

[16] Mulder V., Honey and wax production from *Apis dorsata* and *Apis florea* in submerged *Melaleuca* forests in Vietnam, in: Beetsma J. (Ed.), Bees and Forest in the Tropics, NECTA, Netherlands, 1995, pp. 27–41.

[17] Oldroyd B.P., Smolenski A.J., Cornuet J.M., Wongsiri S., Estoup A., Rinderer T.E., Crozier R.H., Levels of polyandry and intracolonial genetic relationships in *Apis florea*, Behav. Ecol. Sociobiol. 37 (1995) 329–335.

[18] Oldroyd B.P., Smolenski A.J., Cornuet J.M., Wongsiri S., Estoup A., Rinderer T.E., Crozier R.H., Levels of polyandry and intracolonial genetic relationships in *Apis dorsata* (Hymenoptera: Apidae), Ann. Entomol. Soc. Am. 89 (1996) 276–283.

[19] Oldroyd B.P., Clifton M.J., Wongsiri S., Rinderer T.E., Crozier R.H., Polyandry in the genus *Apis*, particularly *Apis andreniformis*, Behav. Ecol. Sociobiol. (1999) in press.

[20] Rinderer T.E., Oldroyd B.P., Wongsiri S., Sylvester H.A., Guzman L.I.D., Potichot S., Sheppard W.S., Buchmann S.L., Time of drone flight in four honey bee species in south-eastern Thailand, J. Apic. Res. 32 (1) (1993) 27–33.

[21] Roberts W.C., Multiple mating of queen bees proved by progeny anf flight tests, Glean. Bee Cult. 72 (1944) 255–259.

[22] Ruttner F., The life and flight activities of drones, Bee World 47 (3) (1966) 93–100

[23] Ruttner F., Woyke J., Koeniger N., Reproduction in *Apis cerana* 1. Mating behaviour, J. Apic. Res. 11 (3) (1972) 141–146.

[24] Ruttner F., Woyke J., Koeniger N., Reproduction in *Apis cerana* 2. Reproductive organs and natural insemination, J. Apic. Res. 12 (1) (1973) 21–34.

[25] Taber S., Factors influencing the circadian flight rhtyhm of drone honey bees, Ann. Entomol. Soc. Am. 57 (1964) 769–775.

[26] Tan N.Q., Some aspects of the reproductive biology and behaviour of the Asiatic giant honeybee, *Apis dorsata* Fabricius, Masters thesis, Universiti Putra Malaysia, 1997.

[27] Tan N.Q., Chinh P.H., Rafter, a traditional technique for enticing *Apis dorsata* colonies to nest and rafter factors affecting the occupation, Int. Conf. on Tropical Bees & the Environment, at Pedu Lake, Kedah, Malaysia, 11–15 March, 1995.

[28] Tan N.Q., Mardan M., Thai P.H., Chinh P.H., Some reproductive biology of *Apis dorsata*, 3rd Asian Apicultural Association Conference, Ha Noi, Vietnam, 6–10 October, 1996.

[29] Tan N.Q., Chinh P.H., Thai P.H., Mulder V., Rafter beekeeping with *Apis dorsata*: Some factors affecting the occupation of rafters by bees, J. Apic. Res. 36(1) (1997) 49–54.

[30] Underwood B.A., Time of drone flight of *Apis laboriosa* Smith in Nepal, Apidologie 21 (1990) 501–504.

[31] Woyciechowski M., Kabat L., Krol E., The function of the mating sign in honey bees, *Apis mellifera* L.: new evidence, Anim. Behav. 47 (3) (1994) 733–735.

[32] Woyke J., Natural and artificial insemination of queen honey bees, Pszcz. Zesz. Nauk 4 (1975) 183–275 (in Polish, English summary).

[33] Woyke J., Natural and artificial insemination of *Apis cerana* in India, J. Apic. Res. 14 (2) (1975) 153–159.

[34] Woyke J., Rearing and instrumental insemination of *Apis florea* queens, in: Connor L.J., Rinderer T., Sylvester H.A., Wongsiri S. (Eds.), Asian Apiculture, Wicwas Press, Cheshire, CT, USA, 1993, pp. 206–209.