Apidologie (1994) 25, 353-360

Original article

The mating flight times of native *Apis cerana japonica* Radoszkowski and introduced *Apis mellifera* L in sympatric conditions

T Yoshida, J Saito, N Kajigaya

Honeybee Science Research Center, Tamagawa University, Machida-shi, Tokyo 194, Japan

(Received 16 June 1993; accepted 21 September 1993)

Summary — The mating flight times of native *Apis cerana japonica* and introduced *A mellifera* were compared in the same biotype in Japan. The queen flight times for *A cerana japonica* and *A mellifera* were 13.15–17.00 h, 12.15–15.00 h, and those of the drones were 13.15–16.30 h, 11.30–15.00 h, respectively. Both the hive departure and mating flight times of *A mellifera* were 1.5–2 h earlier than those of *A cerana japonica*. Successful mating flights of queens occurred between 13.00 and 14.40 h in *A mellifera* and between 14.45 and 16.35 h in *A cerana japonica*.

***A cerana japonica* / mating behavior / mating flight time / *A mellifera* / Japan**

INTRODUCTION

The Asian *Apis cerana* Fabr is a polytypic species distributed from tropical to northern temperate Asia. The origin, route and time of entry of *A cerana* into Japan are not clear, but morphometric and statistical data enabled Ruttner (1988) to classify Japanese *A cerana* as the independent subspecies, *Apis cerana japonica* Radoszkowski. Modern beekeeping in Japan started in 1877 when the western honeybee *Apis mellifera* L was introduced in movable-frame hives.

The mating behavior of *Apis cerana indica* Fabr has been extensively studied (Sharma, 1960; Adlakha, 1971; Ruttner *et al,* 1972; Woyke, 1975; Shah and Shah, 1980; Verma, 1990). The difference in the mating flight time of sympatric *Apis* species is thought to be a key factor of interspecific reproductive isolation (Koeniger and Wijayagunasekera, 1976; Koeniger *et al,* 1988). Intraspecific variation of the flight has also been reported (Rowell *et al,* 1986). In Europe, Ruttner *et al* (1972) and Ruttner and Maul (1983) caught drones of both native *A mellifera* and experimentally introduced *A cerana* at the same time of day in the same drone congregation area. However, there is no detailed information on the interaction between native *A cerana* and introduced *A mellifera* in Asia.

The mating flights of both queens and drones of the 2 species were compared in the same biotype in Tokyo. The drone flights

of *A cerana japonica* were also observed on Tsushima Island where *A mellifera* has not been introduced.

MATERIALS AND METHODS

This study was done in the normal mating season for both honeybee species, from mid-May to late-June from 1989 through 1992. Queen mating flights of *A cerana japonica* and *A mellifera* (basically *ligustica* but it may be mixed with other *mellifera* subspecies) were observed with the aid of a specially designed queen excluder fitted to the entrance of a nucleus hive (Alber *et al*, 1955). The observations were made every day until a mating sign was found 5 d after emergence of the queens. When a queen appeared at the entrance, the excluder was removed to allow her to fly. The returning queen was carefully inspected for the mating sign and was then allowed to enter. Observations of drone flights were recorded in 2 colonies, whose populations each covered at least 6–8 frames of *A cerana japonica* and 10–15 frames of *A mellifera*. Flight time was evaluated by counting outgoing drones for 5 min every 15 or 30 min. The flight durations of marked drones in both species were observed using modified UC Davis-type observation hives (Gary, 1976) with 3–4 frames placed in 12:12 light/dark rooms (light on at 06.30 h and off at 18.30 h) at 25 ± 2°C. The drone flight time of *A cerana japonica* was also observed at Tsushima Island (E 129° 20', N 34° 22'), where *A mellifera* has not been introduced, and at Mine in Yamaguchi Prefecture (E 131° 13', N 34° 12'), which is almost at the same latitude as Tsushima Island. *A cerana japonica* colonies in these areas are kept in traditional bee hives. Two similar colonies placed in the field were examined for 4 d, May 3–6 1992, at Tsushima and for 3 d, May 12–14 1992, at Mine.

Flight times in this report and those reported in the literature were converted to the local time considering longitude of their experimental location.

RESULTS

Queen flights

Queen flights of *A cerana japonica* were observed for 7 d, June 3–6 and June 25–27 1989, and 17 d, May 16–22 and June 21–30, 1990; queen flights of *A mellifera* were observed for 5 d, May 28–June 1, 1989, and 7 d May 8–10 and May 31–June 3 1990. The temperature during the observation days was 23–27°C. A total of 71 flights of 15 queens were observed for *A cerana japonica*, and 73 flights of 13 queens were observed for *A mellifera*. Queens of *A cerana japonica* departed between 13.15 and 17.00 h. Four out of 7 queens in 1989 and 5 out of 8 queens in 1990 returned with the mating sign from 14.45 to 16.35 h. Queens of *A mellifera* departed between 12.15 and 15.00 h. Five out of 8 queens in 1989 and 4 out of 5 queens in 1990 returned with the mating sign from 13.00 to 14.40 h. Since queen flight times observed in 1990 were similar to those in 1989, only the results of 1989 are shown in figure 1, which shows the number of flights and the time until they returned with mating sign. Four *A cerana japonica* queens returned with mating sign compared with 5 *A mellifera* queens. Out of 4 *A cerana japonica* queens, queen No 23 made only 1 flight for 23 min to get the mating sign. Queen No 27 made 3 flights and got the mating sign during the last flight, which took 25 min. Queens No 21A and 21B made 4 flights in 1 day, and both got the mating sign during the last flight. Queen No 21B made a further 2, 1 and 5 flights in 3 consecutive days and returned with the second mating sign during the last flight. *A mellifera* queens No 4, 6 and 19 made 6, 3, and 4 flights, respectively. Queen No 9 made 12 flights in total over 5 d and got the mating sign twice in the last 2 flights of 9 and 5 min duration. Queen No 14 mated twice on days 7 and 8 after emergence.

In the combined results of 1989 and 1990, all 9 *A cerana japonica* queens with mating sign returned later than 14.45 h and 9 *A mellifera* queens with mating sign returned before 14.40 h.

The flight duration of *A cerana japonica* queens ranged from 30 s to 37.4 min.

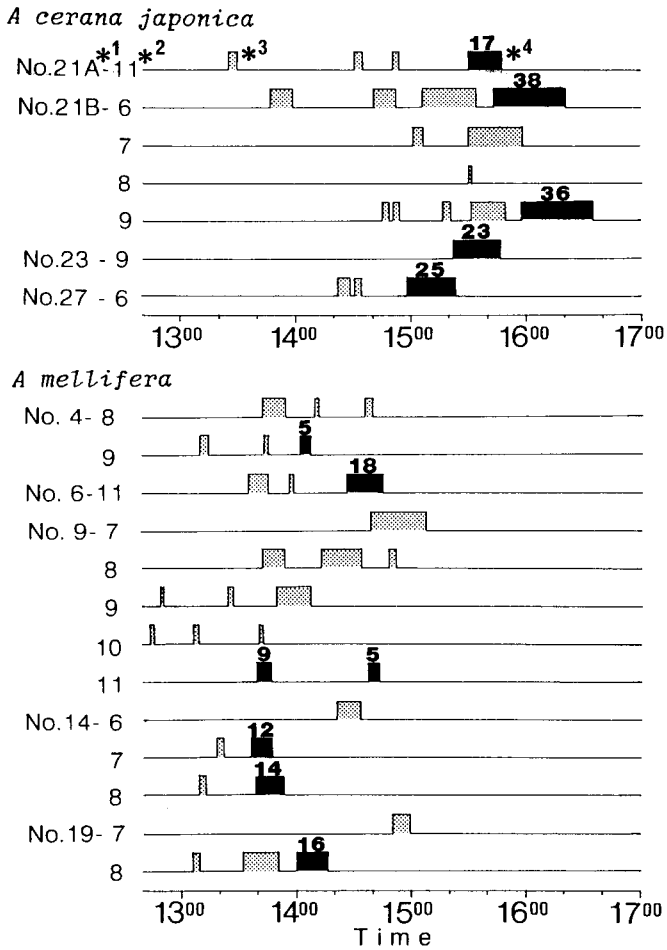

Fig 1. Queen flight times of *A cerana japonica* and *A mellifera*: *1 queen number; *2 age of queens; *3 dotted square shows duration of flight without mating sign; *4 solid square shows duration (min) of flights with mating sign.

The mating flight (with mating sign) was relatively long, ranging from 16.5 and 37.4 min with an average of 26.3 min (table I). Flights shorter than 15 min were thought to be orientation flights. In *A mellifera*, flights with a mating sign took between 5 and 18.2 min with an average of 11.7 min. This is significantly shorter than mating flights of *A cerana japonica*. Orientation flights in *A mellifera* were estimated to take 5 min or less (table I).

The average age of queen at the first flight was 9.8 d for *A cerana japonica* and 8.3 d for *A mellifera*. The average age of a queen who returned with mating sign was 12.4 and 10.4 d, respectively, but these values were not statistically different (table II).

Table I. Duration of flights by queens of *A cerana japonica* and *A mellifera*.

Duration (min)	*No of fights observed*					
	A cerana japonica			A mellifera		
	Total	*Without mating sign*	*With mating sign*	*Total*	*Without mating sign*	*With mating sign*
0 ~ 5	27	27	0	36	35	1
~ 10	11	11	0	19	14	5
~ 15	7	7	0	8	6	2
~ 20	7	6	1	8	4	4
~ 25	9	5	4	1	1	0
~ 30	6	4	2	1	1	0
~ 35	1	1	0			
~ 40	3	0	3			
Total	71	61	10	73	61	12
Average flight time (min) ± SD	12.1 ± 10.0	9.8 ± 8.4	26.3 ± 6.5	7.0 ± 5.9	6.1 ± 5.9	11.7 ± 5.0

Table II. Age of queen at the first flight and the flights with mating sign of *A cerana japonica* and *A mellifera*.

Flight	*Age of queen (d)*			
	A cerana japonica		A mellifera	
	Range	*Mean ± SD*	*Range*	*Mean ± SD*
First flight	6–14 (n = 15)	9.8 ± 2.6	6–11 (n = 13)	8.3 ± 2.1
Flight with mating sign	6–24 (n = 9)	12.4 ± 5.2	7–17 (n = 9)	10.4 ± 2.8

Drone flights

Drone flights of *A cerana japonica* were observed for 3 d, May 10–12 1991, and drone flights of *A mellifera* for 3 d, May 26–28 1991. *A cerana japonica* drones departed from 13.15 to 16.30 h with a peak from 15.00 to 15.30 h. The range in *A mellifera* was from 11.30 to 15.00 h with a peak from 13.00 to 13.30 h (fig 2). The drone flight time of *A cerana japonica* was about 2 h after that of sympatric *A mellifera*.

The flight duration of 84 marked *A cerana japonica* drones 6–10 d old, and of 85 marked *A mellifera* drones 9–16 d old were observed. *A cerana japonica* drones 8–9 d old and *A mellifera* drones 14–15 d old flew most frequently. A flight duration of less than 10 min was frequent in *A cerana japonica* whereas it was 10–20 min in *A mellifera*.

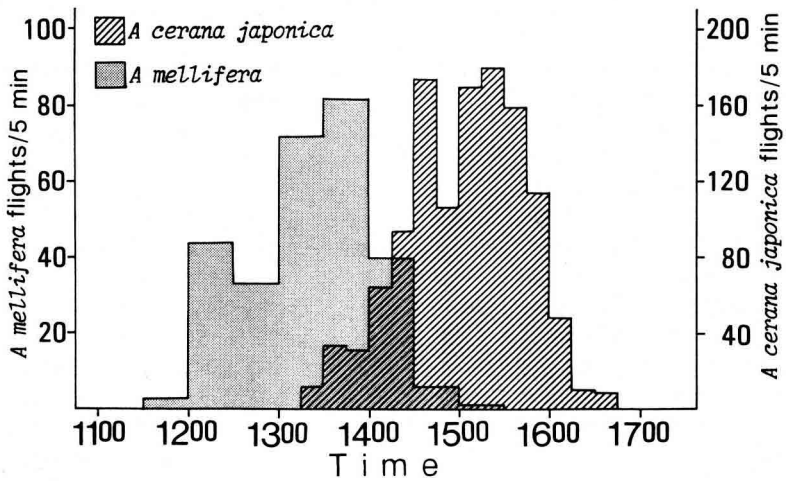

Fig 2. Drone flight times of *A cerana japonica* (▨) and *A mellifera* (▩).

The longest drone flight in *A cerana japonica* was 49 min whereas it was 36 min in *A mellifera* (fig 3).

Drones in Tsushima Island departed from 12.45 to 16.15 h, whereas those in Yamaguchi departed from 13.30 to 16.30 h, with the peak from 14.45 to 15.45 h; there is no significant difference between these times and those observed in Tokyo (fig 4). Dawn in Tsushima and Yamaguchi is 50 min and 35 min later, respectively, than in Tokyo (E 139° 26', N 35° 35'), but this time difference did not influence the drone flight time.

DISCUSSION

A cerana indica queen mating flights are reported to take place between 13.26 and 14.26 h (Woyke, 1975) local time. They occur between 12.40 and 13.40 h in India (Verma, 1990), and between 16.07 and

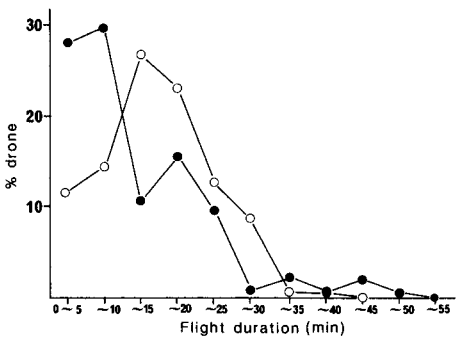

Fig 3. Duration of flight by drones of *A cerana japonica* (●, n = 128) and *A mellifera* (O, n = 138).

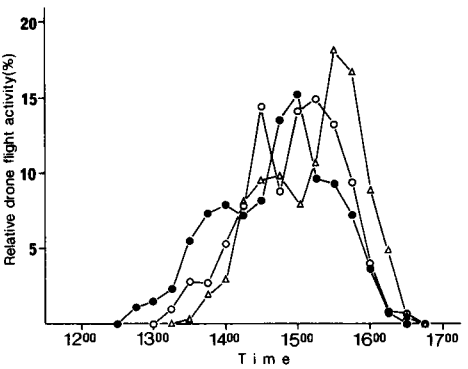

Fig 4. *A cerana japonica* drone flight time in 3 places: Tokyo (O, n = 1 207); Tsushima (●, n = 5 965); and Yamaguchi (Δ, n = 2 976).

16.47 h in Sri Lanka (Punchihewa *et al,* 1990). In our experiments, *A cerana japonica* queens made mating flights between 15.03 and 16.18 h and took longer than *A cerana indica* queens. Mating flights of *A cerana indica* drones were reported between 16.07 and 17.07 h in Sri Lanka (Koeniger and Wijayagunasekera, 1976), between 15.22 and 16.52 h in Sri Lanka (Punchihewa *et al,* 1990), between 13.29 and 15.14 h in Borneo (Koeniger *et al,* 1988), and between 13.25 and 14.55 h in India (Verma, 1990). Drone flights of *A cerana japonica* occurred between 13.33 and 16.48 h and took longer than those of *A cerana indica* drones.

The time, number and length of *A mellifera* queen flights have been observed by many researchers. Mating flights occur only between 12.00 and 17.00 h with the greatest frequency between 14.00 and 16.00 h (Ruttner, 1956). Koeniger *et al* (1989) reported that queens of *A mellifera carnica* and *A mellifera ligustica* in Austria mated between 13.59 and 16.01 h, and 15.24 and 16.16 h, respectively. In Japan, *A mellifera* queen mating flights occurred between 13.18 and 14.48 h, which is earlier than in other reports. In Louisiana, the average flight time of *A mellifera* drones is between 13.56 and 16.26 h, but they flew almost 2 h earlier early in the mating season (Taber, 1964). In Japan, the flight time of *A mellifera* drones was between 11.48 and 15.18 h during the peak season, which tends to be earlier than the mating flight time of queens.

For the difference in the mating flight time, Koeniger and Wijayagunasekera (1976) and Koeniger *et al* (1988) report 3 different daily drone flight periods for the 3 sympatric species in Sri Lanka and Borneo. The duration of the mating flights of *A cerana japonica*, which is the only native honeybee in Japan, is longer than the duration in southeast Asia where there are other native honeybee species. It seems that the mating flight time in Japan is not severely restricted because there has been no sympatric *Apis* species until recent introduction of *A mellifera.*

A cerana drones introduced from Pakistan and China to Europe showed high flight activity at the same time of day as *A mellifera* (Ruttner *et al,* 1972; Ruttner and Maul, 1983). Hoshiba *et al* (1981) also state (without evidence) that mating flights of *A mellifera* and *A cerana japonica* occur at the same time in Japan. In the present observations in Tokyo, however, we found a clear time difference: both departure and mating flight times of *A cerana japonica* were 1.5–2 h later than those of *A mellifera.* On Tsushima Island, where *A mellifera* has never been introduced, drones flew at almost the same timing as in Tokyo. This suggests that the observed chronological separation in mainland Japan where *A mellifera* coexists is due to the difference of their own timings developed in allopatric conditions. In other words, interspecific chronological selection to separate the 2 species does not seem to have been operating during this 100 years of coexistence.

It seems probable that the observed difference in the timing of reproductive behavior takes part in the actual reproductive isolation, even if it resulted by chance, together with the difference in drone congregation area. The conditions for *A mellifera* drone congregation areas in Japan (first found by Yoshida, 1990) were similar to those described in Germany (Ruttner, 1973; Koeniger *et al,* 1979), but for *A cerana japonica* they were different. This species congregates above the crown of tall trees which are visually distinct (Yoshida and Yamazaki, 1993).

In spite of the reproductive isolation tendency, however, interspecific copulation or copulatory attempts may occur in natural conditions. Ruttner and Maul (1983) found an *A cerana* queen with an *A mellifera* mating sign. We have also confirmed that a fixed *A cerana japonica* queen does attract *A mellifera* drones and can copulate with

them in *A mellifera* drone congregation areas (Yoshida and Yamazaki, 1993). A much more systematic survey in this respect is in progress in our institute.

ACKNOWLEDGMENTS

We thank N Koeniger and G Koeniger, Institut fur Bienenkunde, Universitat Frankfurt aM, who advised and helped us in various ways. We are also grateful to M Matsuka and M Sasaki, Tamagawa University for their useful suggestions.

Résumé — Horaires des vols de fécondation de l'abeille indigène *Apis cerana japonica* Radoszkowski et de l'abeille introduite *Apis mellifera* L en conditions sympatriques. On a comparé les vols de fécondation des reines et des mâles de l'abeille indigène *Apis cerana japonica* et de l'abeille introduite *Apis mellifera* dans le même biotope au Japon. Les reines d'*A c japonica* s'envolaient entre 13 h 15 et 17 h, celles d'*A mellifera* entre 12 h 15 et 15 h. Le retour des reines avec signe de fécondation s'effectuait entre 14 h 45 et 16 h 35 chez *A c japonica* et entre 13 h et 14 h 40 chez *A mellifera* (fig 1). Pour les mâles, les vols avaient lieu entre 13 h 15 et 16 h 30 avec un pic de 15 h à 15 h 30 chez *A c japonica* et de 11 h 30 à 15 h avec un pic de 13 h à 13 h 30 chez *A mellifera* (fig 2). Un vol de fécondation durait en moyenne 26,3 min chez *A c japonica* et 11,7 min chez *A mellifera* (tableau I). L'âge moyen des reines rentrant avec un signe de fécondation était de 12,4 j pour *A c japonica* ($n = 9$) et de 10,4 min pour *A mellifera* ($n = 9$) (tableau II). Une durée de vol inférieure à 10 min se rencontrait fréquemment chez les mâles d'*A c japonica*, tandis que chez les mâles d'*A mellifera* la plupart durait de 10 à 20 min (fig 3). Lors de ces observations faites à Tokyo, les horaires d'envol et les horaires des vols de fécondations d'*A c japonica* se situaient 1 h et demie à 2 h plus tard que ceux d'*A mellifera*, contrairement aux résultats précédents qui indiquaient la même période de vol pour les 2 espèces (Ruttner *et al*, 1972; Ruttner et Maul, 1983). Sur l'île de Tsushima, où *A mellifera* n'a jamais été introduite, les mâles effectuaient leurs vols presque à la même heure que ceux de Tokyo (fig 4). Cela laisse supposer que la séparation chronologique observée dans l'île principale du Japon, où *A mellifera* et *A c japonica* coexistent, est due à la différence de leurs propres horaires qui se sont mis en place en conditions allopatriques. Une sélection chronologique interspécifique pour séparer les 2 espèces ne semble pas avoir fonctionné au cours des 100 années de coexistence.

***A cerana japonica* / vol de fécondation / accouplement / *A mellifera* / Japon**

Zusammenfassung — Paarungszeit der einheimischen *A cerana japonica* Radoszkowski und der eingeführten *A mellifera* L unter sympatrischen Bedingungen. Die Paarungsflüge von Königinnen und Drohnen beider Arten, der ursprünglichen *A cerana japonica* und der eingeführten *A mellifera*, wurden im selben Biotop miteinander verglichen. Königinnen von *A cerana japonica* starteten ihre Paarungsflüge zwischen 13.15 und 17.00 Uhr, *A mellifera* Königinnen zwischen 12.15 und 15.00 Uhr. Die Flugzeit der Königinnen, die mit einem Begattungszeichen zurückkehrten, lag zwischen 14.45 und 16.35 Uhr bei *A cerana japonica*, bei *A mellifera* zwischen 13.00 und 14.40 Uhr (Abb 1). Bei den Drohnenflügen lag die Zeitspanne zwischen 13.15 und 16.30 Uhr, mit einem Peak zwischen 15.00 und 15.30 Uhr bei *A cerana japonica*, bei *A mellifera* zwischen 11.30 und 15.00 Uhr, mit einem Peak von 13.00 und 13.30 Uhr (Abb 2). Die durchschnittliche Flugdauer betrug 26,3 min bei *A cerana japonica* und 11,7 min bei *A mellifera* (Tabelle I). Das Durchschnittsalter der Königinnen, die mit einem Begattungszeichen

zurückkehrten, betrug 12,4 Tage bei *A cerana japonica* (n = 9) bzw 10,4 Tage bei *A mellifera* (n = 9) (Tabelle II). Die Dauer der Drohnenflüge lag bei *A cerana japonica* häufig unter 10 min, während bei *A mellifera* Zeiten zwischen 10 und 20 min überwogen (Abb 3). Bei den hier beschriebenen Beobachtungen in Tokio lagen sowohl die Abflugs- als als auch die Paarungszeiten von *A cerana japonica* 1,5 bis 2 Stunden später als die von *A mellifera*. Bei früheren Beobachtungen in Deutschland flogen beide Arten zur selben Zeit (Ruttner *et al*, 1972; Ruttner und Maul, 1983). Auf den Tsushima Inseln, auf die *A mellifera* nie eingeführt wurde, flogen die Drohnen etwa zur gleichen Zeit wie in Tokio (Abb 4). Das legt den Schluß nahe, daß die beobachtete zeitliche Trennung der Paarungsflüge auf der Hauptinsel von Japan zwischen den beiden Arten wahrscheinlich auf die ursprünglichen Zeitmuster zurückzuführen sind, die sich unter allopatrischen Bedingungen entwickelt haben. Eine Selektion auf reproduktive Isolation beider Arten durch unterschiedliche Paarungszeiten hat sich während 100 Jahren der Koexistenz wahrscheinlich nicht auswirken können.

***A cerana japonica* / Paarungsflug / Paarungsverhalten / *A mellifera* / / Japan**

REFERENCES

Adlakha RL (1971) Preliminary studies on the mating behavior of *Apis mellifera* and *Apis indica* queens and their age of starting oviposition. *Proc Int Beekeep Congr* 23, 420-425

Alber M, Jordan R, Ruttner F (1955) Von der Paarung der Honigbiene. *Z Bienenforsch* 3, 1-28

Gary NE (1976) How the construct and maintain an observation bee hive. Division of Agricultural Science, Univ California Leaflet 2853

Hoshiba H, Okada I, Kusanagi M (1981) The diploid drone of *Apis cerana japonica* and its chromosomes. *J Apic Res* 20, 143-147

Koeniger G, Koeniger N, Fabritius M (1979) Some detailed observations of mating in the honeybee. *Bee World* 60, 53-57

Koeniger G, Koeniger N, Pechhacker H, Ruttner F, Berg S (1989) Assortative mating in a mixed population of European honeybees, *Apis mellifera ligustica* and *Apis mellifera carnica*. *Insectes Soc* 36, 129-138

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

Punchihewa RWK, Koeniger N, Koeniger G (1990) Mating behaviour of *Apis cerana* in Sri Lanka. *In: Social Insects and the Environment IUSSI India* (Veeresh GK, Mallik B, Viraktamath CA, eds) Oxford and JBH Publ Co ltd, New Dehli, 108

Rowell GA, Taylor OR, Locke S (1986) Variation in drone mating times among commercial honey bee stocks. *Apidologie* 17, 137-158

Ruttner F (1956) The mating of the honeybee. *Bee World* 37, 3-15

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

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

Ruttner F, Maul V (1983) Experimental analysis of the reproductive interspecific isolation of *Apis mellifera* L and *Apis cerana* Fabr. *Apidologie* 14, 309-327

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

Shah FA, Shah TA (1980) Early life, mating and egg laying of *Apis cerana* queens in Kashmir. *Bee World* 61, 137-140

Sharma PL (1960) Observations on the swarming and mating habits of the Indian honeybee. *Bee World* 41, 121-125

Taber S III (1964) Factors influencing the circadian flight rhythm of drone honey bees. *Ann Entomol Soc Am* 57, 769-775

Verma LR (1990) Beekeeping in Integrated Mountain Development. Oxford & IBH Publishing New Delhi

Woyke J (1975) Natural and instrumental insemination of *Apis cerana indica* in India. *J Apic Res* 14, 153-159

Yoshida T (1990) Drone congregation area of *Apis mellifera* first discovered in Japan. *Honeybee Science* 11; 161-162 (in Japanese)

Yoshida T, Yamazaki M (1993) Difference in drone congregation areas of *Apis mellifera* and *A cerana japonica* as a reproductive isolation mechanism. *In: Asian Apiculture, Proc First Symposium on Asian Honey Bee and Bee Mites, Bangkok Thailand* (LK Conner, T Rinderer, A Sylvester, S Wongsiri, eds) Wicwas Press, 99-103