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Time of drone flight in four honey bee species in south-eastern Thailand[1]

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SUMMARY

At Chanthaburi, Thailand, four species of *Apis*, *A. andreniformis*, *A. florea*, *A. cerana* and *A. dorsata*, are sympatric. Observations were carried out on three wild colonies of each species on various days in February 1992. The daily drone flight periods were only partially specific: *A. andreniformis* from 12.15 h to 13.45 h; *A. florea* from 14.00 h to 16.45 h; *A. cerana* from 15.15 h to 17.30 h; and *A. dorsata* from 18.15 h to 18.45 h. The significance of these partially separate drone flight periods is discussed in terms of both reproductive isolation and evolution.

Keywords: drone honey bees, *Apis andreniformis*, *Apis cerana*, *Apis dorsata*, *Apis florea*, flight, periodicity, reproductive isolation, sympatric species, evolution, Thailand

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INTRODUCTION

Reproductive isolation is a fundamental requirement for the definition of species (Mayr, 1976). Evidence for the maintenance of reproductive isolation between currently sympatric species of the genus *Apis* is generally of two types. First, the male genitalia (endophallus) of each species of honey bee are distinct (Koeniger *et al.*, 1991; Ruttner, 1988). In general, the honey bee drone has an endophallus having various cornuae, hairy fields and lobes. However, this general theme has evolved into highly elaborate and species-specific forms (Koeniger *et al.*, 1991; Ruttner, 1988). Presumably, endophallus differences would prevent mating between drones of one species and queens of another. However, such attempts to mate are probably rare since the second mechanism providing reproductive isolation between sympatric species is the time of drone flight (Ruttner, 1988). Honey bees mate in flight. Drones and queens leave the colony at approximately the same time of day, meet in the air, and mate. Synchronous gyne flight assures mating and reduces the risk of predation (Moritz, 1985). Observations have been made of drone flight times in Sri Lanka of *A. cerana*, *A. florea* and *A. dorsata* (Koeniger & Wijayagunasekera, 1976) and in Sabah (north-east Borneo) of *A. andreniformis*, *A. cerana*, *A. dorsata* and *A. koschevnikovi* (Koeniger *et al.*, 1988; Mathew & Mathew, 1990). In general, these observations suggest that each naturally occurring species in a biotope has a specific separate time period each day in which mating flights occur. Queens take mating flights at similar times as drones of the same species (Koeniger, 1991). If this tendency for synchronized mating flights is widespread, temporal differences in the flight times of reproductive castes of different species are a complete reproductive isolating mechanism.

At least four species of *Apis* facilitate mate location by queens producing 9-oxodec-*trans*-2-enoic acid as a component of a mate-attractant pheromone (Shearer *et al.*, 1970). Trans-specific attraction has been demonstrated for *A. mellifera* drones to pheromone extracts of *A. cerana* and *A. florea* (Butler *et al.*, 1967). This suggests that *Apis* generally share this mate-attraction mechanism and that species-specific mating flight times are critical in reducing cross-species attraction during mating flights. Although differences in endophallus morphology and embryonic development (Ruttner, 1988) assure reproductive isolation, species-specific flights contribute to optimum mating efficiency.

Recently, *A. andreniformis* has been recognized as a true biological species (Wu & Kuang, 1986, 1987; Wongsiri *et al.*, 1990). It, like *A. florea*, is a dwarf honey bee that lives on a single comb attached to branches, usually in thick shrubs. Indeed, *A. andreniformis* is sufficiently similar to *A. florea* that until only recently they were thought to be forms of the same species (Ruttner, 1988). We studied the drone flight times of four species of naturally sympatric *Apis* (*A. andreniformis*, *A. florea*, *A. cerana* and *A. dorsata*) in south-east Thailand. This is the first report concerning the drone flight times of sympatric *A. andreniformis* and *A. florea*.

MATERIALS AND METHODS

Observations were carried out from 23 February to 29 February 1992 on honey bee colonies on or near the Chanthaburi Horticultural Research Centre of the Thai Department of Agriculture near Chanthaburi, Thailand. Most colonies were within 0.5 km of each other. One *A. andreniformis* colony (2a) (table 1) was 1 km distant from the centre of the main group of colonies. Two *A. dorsata* colonies (2d and 3d) were 0.5 and 5 km distant, respectively.

Three colonies each of *A. andreniformis*, *A. florea*, *A. cerana* and *A. dorsata* were observed on various days (table 1). On 27 February, three colonies each

TABLE 1. Dates in February 1992 of observations of drone flight times for several colonies of four species of honey bee[1].

A. andreniformis	*A. florea*	*A. cerana*	*A. dorsata*
Colony 1a: 25, 26, 27, 28	Colony 1f: 23, 24, 26, 27, 28	Colony 1c: 24	Colony 1d: 23, 24, 27
Colony 2a: 26, 27	Colony 2f: 27	Colony 2c: 26, 27	Colony 2d: 27
Colony 3a: 27	Colony 3f: 27	Colony 3c: 29	Colony 3d: 25
Total colony days **4**	**4**	**3**	**5**

[1]Rain or clouds prevented drone flight on 23, 26, and 28 February except for *A. dorsata* drones from colony 1d which flew on 23 February.

of *A. andreniformis* and *A. florea* , one colony of *A. cerana* and two colonies of *A. dorsata* were observed.

All colonies were wild colonies found in the area and were sufficiently accessible to permit drones to be clearly observed. The *A. andreniformis* and *A. florea* colonies were located in trees or shrubs that could either be seen closely by climbing the tree, using a ladder, or observed from the ground. One *A. cerana* colony was in a termite nest that was relocated from about 8 km away. The other *A. cerana* colonies were in the walls of a house and could be observed from the ground. *A. dorsata* colonies were either on a water tower (25 m) with a built-in ladder, or in trees (5 m and 4 m) where nests could be observed from ladders.

For *A. andreniformis*, *A. florea* and *A. cerana*, both outgoing drones and incoming drones were counted for each minute. Based on preliminary examinations, observations started at least 30 min prior to any drone flight for all colonies. For these three species, observations were pooled for 15 min periods by adding all incoming and outgoing flights together. *A. andreniformis* and *A. florea* incoming drones were especially easy to detect. Outgoing drones flew faster than incoming drones and simultaneous departures were difficult to count. Combining counts produces a more accurate representation of the flight time periods. For *A. dorsata*, on one occasion (colony 1d, 27 February), an attempt was made to estimate numbers of drones during one 20 s period for each minute of the interval having drone flights. This was very difficult since hundreds of drones flew within a few minutes. Other observations of *A. dorsata* recorded the times of the beginning, ending and peak period of drone flight.

Times were recorded in standard time. For analysis and interpretation, 12 min were added to each time to adjust 12.00 h to coincide with the sun's zenith in accordance with the dates of observation and the latitude and longitude of Chanthaburi, Thailand. No substantial variation in drone flight time was noted for any species or between days for the same species. Consequently, all data from all colonies of a species and all days (excluding rainy or cloudy days not having drone flight) were pooled for presentation. Certain colonies were analysed individually to illustrate obvious groupings of flight within the overall drone flight period.

RESULTS

Apis andreniformis

The colonies observed had adult drones but did not have drone brood or drone cells in their combs. Thus, the drones were mature drones that came with the colonies to relatively new nesting sites. Such drones can be expected to take mating flights rather than just orientation flights as young drones of *A. mellifera* are known to do (Witherell, 1971).

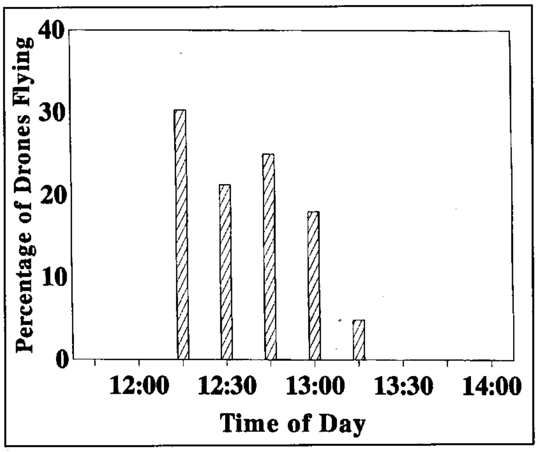

FIG. 1. Pooled daily flight of *Apis andreniformis* drones. Data are presented as a percentage of the total outgoing and incoming flights within 15-min periods made by drones from three colonies, for a total of 4 colony days (see table 1).

Drones were hard to detect among the bees of the protective curtain at any time other than during, or somewhat before, the time of drone flight. Apparently, drones of *A. andreniformis* spend most of their time under the protective curtain. During the half-hour before drone flight began, drones appeared on the protective curtain and walked upward. They began flights from the honey storage area at the crown of the nest. Drone flight did not occur on cloudy days. On sunny days, drone flight began at 12.15 h and continued to 13.15 h. Drone flight time was distributed around a peak time of about 12.45 h (fig. 1). During this short period, individual drones tended to leave with small groups of 2–10 drones at intervals of 2–7 min. Some drones performed movements reminiscent of the round dance of foraging workers (Rinderer *et al.*, 1992). These drone 'dances' seemed to facilitate group flights. Group flights are evident when the data are considered in one-minute intervals for colony 1a on 25 February (fig. 2). This grouping was evident in the counts of both the outgoing and incoming flights.

Apis florea

Evidently *A. florea* colonies have a reproductive cycle which has a somewhat different annual timing from that of *A. andreniformis*. The majority of *A. florea* colonies that we observed in the area and all of the *A. florea* colonies that we observed to determine drone flight time were clearly in the general process of colony reproduction. Multiple swarm cells, large areas of nests having drone cells, and adult drones were common in *A. florea* colonies in the area, as

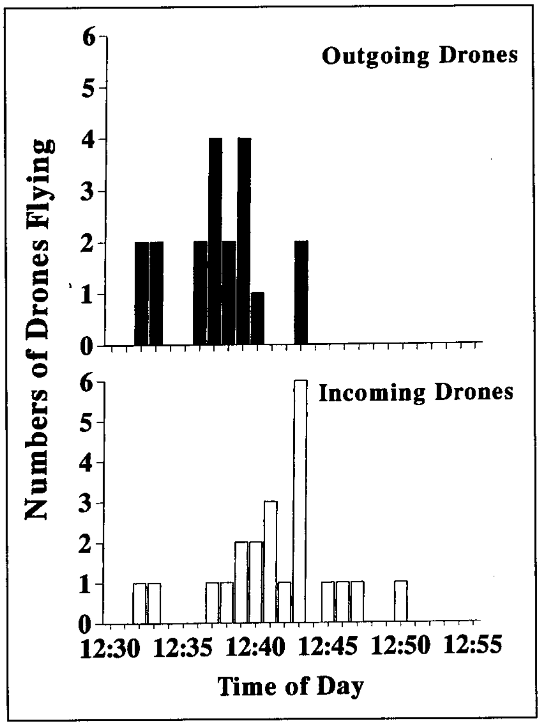

FIG. 2. The flight of *Apis andreniformis* colony 1a (table 1) on 25 February. Data are presented as actual numbers observed in 1-min periods. The dark bars indicate outgoing drone flights and the light bars indicate incoming drone flights.

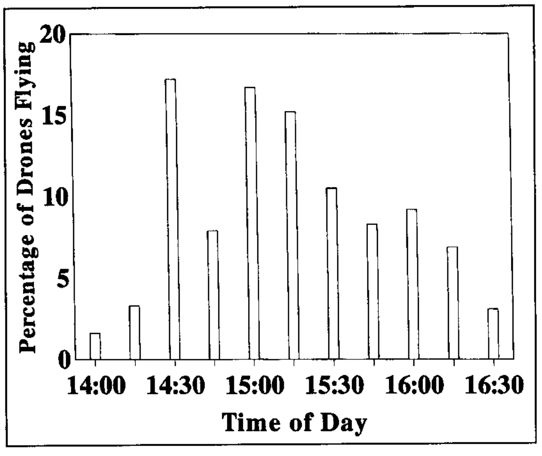

FIG. 3. Pooled daily flight of *Apis florea* drones. Data are presented as a percentage of the total outgoing and incoming flights within 15-min periods made by drones from three colonies, for a total of 4 colony days (see table 1).

were colonies that had recently swarmed. The colonies we observed all had multiple queen cells and numerous drones.

Drones were equally hard to detect in colonies of *A. florea*, other than between just shortly prior to the drone flight period to shortly after the period. The interval before drone flight for *A. florea* was about an hour longer than it was for *A. andreniformis*. Like *A. andreniformis*, *A. florea* drones evidently spend the majority of their time under the protective curtain. They also walked upward and flew from the honey storage area of the nest. The *A. florea* drone flight period was from 13.45 h to 16.45 h on sunny days only. A peak of flight occurred at 15.15 h (fig. 3).

A. florea drones also left on and returned from mating flights in groups. The intervals between these bouts of flights were about 30 min (fig. 4). Again, counts of both the outgoing and the incoming flights show this grouping.

Apis cerana

Like *A. florea*, *A. cerana* was observed during its swarming season. Caste swarms were evident in the area and one colony being observed for drone flight times swarmed during observation (drone flight data from this colony for this day were not included in the analysis since swarming probably produced a rhythm of flight activity not typical for mating).

A few early drone flights were observed between 14.00 h and 15.15 h. A minor flight peak that was comprised of drones that left and returned during the same minute was seen around 15.45 h. The main drone flight occurred between 15.45 h and 17.45 h with a peak at about 16.30 h (fig. 5). Groups of drones flying together may have occurred for two of the three colonies but were not evident in the third (fig. 6).

Apis dorsata

It was not determined whether or not the *A. dorsata* population was in a swarming season. However, of the eight colonies we observed, all had adult drones present.

Drone flight was after sunset between about 18.45 h and 19.00 h. Flight rapidly increased to a peak intensity mid-way in the period (fig. 7). It was not clear whether or not *A. dorsata* drones flew in small groups during the overall period. However, it is doubtful since the overall duration was so short.

DISCUSSION

The periods of drone flight time for the four species were generally well separated (fig. 8). Assuming that the queens of each species flew at the same time as the drones of the same species, there would be little chance of drones from one species being in flight at the same time as queens of another species, in most cases.

However, this is not clearly so from the drone flight data of *A. florea* and *A. cerana*. Even assuming that the earlier peak of drone flight of *A. cerana* around 15.45 h is an orientation flight, many *A. florea* drone flights fall within the flight period of *A. cerana*, and many of the drone flights of *A. cerana* fall within the flight period of *A. florea*. Although these data do not show unambiguous separation of mating flight times, it is still possible that flight time differences enhance reproductive isolation. The peak time of flight for the drones of *A. cerana* does not occur during the period of drone flight time overlap, assuming that the earlier flights of *A. cerana* drones are indeed orientation flights. If *A. florea* queens fly within a more limited period which coincides with the peak of the *A. florea* drone flight, then reproductive isolation through separation of flight times would still occur for *A. florea*. Some *A. florea* drones were still flying during the peak period of *A. cerana* drone flight time and thereby also during the most likely time of *A. cerana* queen flight times (Woyke, 1975). Perhaps the *A. florea* drones that are returning to their colonies by that time are not likely to encounter *A. cerana* queens, or perhaps reproductives of *A. florea* are not attracted to the congregation areas in tree canopies used by *A. cerana* (Punchihewa *et al.*, 1990; Koeniger, 1991). However, because of the similarity of the mate attraction pheromones, it is likely that some trans-specific attraction reduces the mating efficiency of both *A. florea* and *A. cerana*.

Our observations of the reproductive status of colonies suggest that some reproductive isolation may be achieved in *Apis* by differential seasonal timing in swarming and hence queen production between species. Colonies, especially healthy ones, may maintain drones through much of the year. However, except during swarming, queens are only produced in the replacement of lost or failing queens. Such a differential appears to be operating in the Chanthaburi area with the development of *A. andreniformis* colonies following a different timing from that of *A. florea* colonies. This differential reinforces the complete reproductive isolation between *A. andreniformis* and *A. florea*.

The flight times of *A. andreniformis* are similar to those reported for *A. andreniformis* in Chiang Mai province in Northern Thailand (personal observations, SW), peninsular Malaysia (personal communication, Makhdzir Mardan) and East Malaysia by Mathew and Mathew (1990). Mathew and Mathew indicate that the drones they observed started to fly at 11.30 h. However, as standard time in East Malaysia is adjusted to the time zone of peninsular Malaysia, the sun was probably past its zenith at the time of first drone flight. This may also be the case

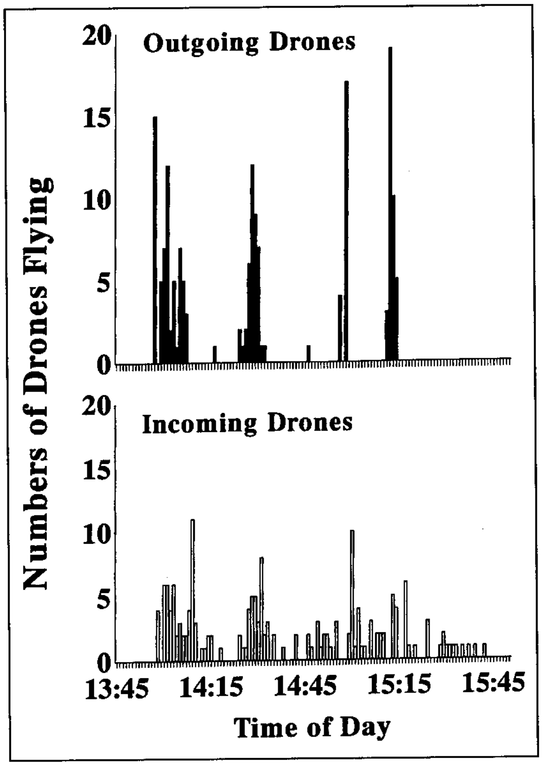

FIG. 4. The flight of *Apis florea* colony 1f (table 1) on 24 February. Data are presented as actual numbers observed in 1-min periods. The dark bars indicate outgoing flights and the light bars indicate incoming drone flights.

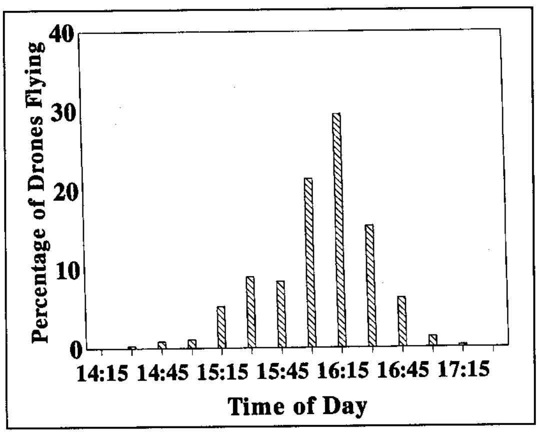

FIG. 5. Pooled daily flight of *Apis cerana* drones. Data are presented as a percentage of the total outgoing and incoming flights within 15-min periods made by drones from three colonies, for a total of 3 colony days (see table 1).

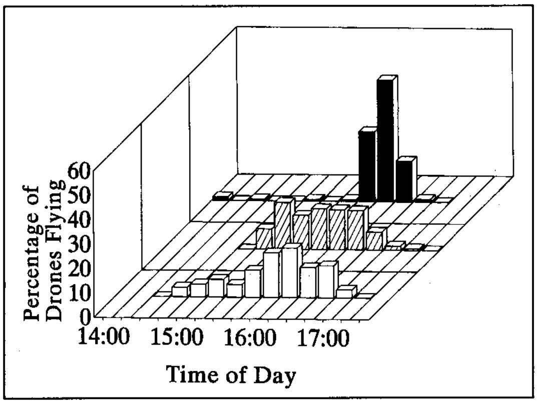

FIG. 6. The separate drone flights of all three colonies of *Apis cerana*. Data are presented as a percentage of total incoming and outgoing flights recorded for each 15-min period. Each bar type represents the flight of a different colony.

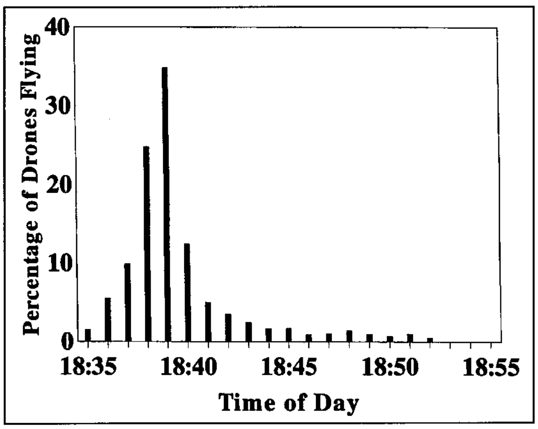

FIG. 7. Daily flight of *Apis dorsata* drones. Data are presented as a percentage of the total outgoing and incoming flights estimated for 1-min periods made by drones from colony 1d on 27 February (see table 1). Other observations of *A. dorsata* colonies produced similar results.

for the few *A. florea* drone flights observed as occurring before 12.00 h in Sri Lanka by Koeniger and Wijayagunasekera (1975). Orientation from polarized light is interfered with at the sun's zenith (von Frisch, 1967). Perhaps a general characteristic of *Apis* is that mating flights are taken in the afternoon.

It is interesting that *A. andreniformis* and *A. florea* drones leave and return in groups. It may be that this behaviour facilitates mate finding, mating, or survival, by avoiding predation (Moritz, 1985) or improved orientation on returning to the nest. Honey bees produce haploid males. In most circumstances all the drones in a nest can be expected to be produced by the same queen and to have a coefficient

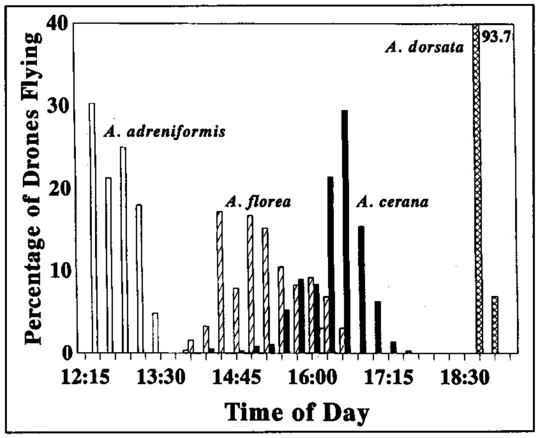

FIG. 8. Daily flight of drones of four *Apis* species. From figs 1, 3, 5 and 7.

of relationship of 0.5. Genetically, drones are gametes of the queen and may compete with other groups of drones in ways parallel to the competition of spermatozoa from different males generally (Parker, 1970) and specifically in honey bees (Harbo, 1990).

Evidently, the drone flight time of *A. florea* is quite variable across its range. Its initiation time, peak time, and ending time are all about one hour earlier in Sri Lanka (Koeniger & Wijayagunasekera, 1975). In SE Thailand, *A. florea* drones have a later flight period of 14.00 h–16.45 h. Interestingly, the duration of the drone flight period of *A. florea* is about 3 h in both Sri Lanka and SE Thailand. This extended period does not interfere with reproductive isolation in Sri Lanka but may do so in SE Thailand.

This imperfect separation of drone flight times suggests that *A. cerana* may be the honey bee which has most recently arrived in SE Thailand. *A. florea* and *A. andreniformis* have well separated drone flight times in SE Thailand, suggesting a long cohabitation of the area and the operation of natural selection. *A. florea* and *A. cerana* have flight times that are different in Sri Lanka, again suggesting long cohabitation and the effects of selection. A similar complete separation of flight times between *A. florea* and *A. cerana* has not happened in SE Thailand. This observation suggests that less time has been available for natural selection to affect the interactions of *A. cerana* in SE Thailand than was available for *A. cerana* in Sri Lanka.

The only other report of an overlap of flight times for drones of sympatric species is for *A. koschevnikovi* and *A. dorsata* in E Malaysia (Koeniger *et al.*, 1988). This overlap is only for a few minutes and does not include the central portion of the drone flight period of either species. Thus, it is probably less consequential than the wide overlap of *A. florea* and *A. cerana* in SE Thailand.

The conjecture that *A. cerana* is the most recent honey bee arrival in SE Thailand suggests the hypothesis that the cavity nesting honey bee *A. cerana* evolved in isolation in SW Asia and later spread throughout Asia into ranges already occupied by the exposed comb species. Arguing from thermoregulation considerations, Ruttner (1988) reached the same general hypothesis, naming the Himalayas as the most likely SW Asian region to provide the conditions that would support the evolution of cavity nesting honey bees. If cavity nesting honey bees did evolve in SW Asia, then open nesting honey bees are probably the more ancient species as suggested by Ruttner (1988) rather than the cavity nesting bees as Koeniger (1976) suggests. This and other questions on the history and evolution of *Apis* may be better understood by an examination and comparison of drone flight times in many locations.

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