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Mating behavior in honey bees (Genus *Apis*)

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Annual mating periods

Monogyny is one of the basic features of the honeybee colony. This links the rearing of new queens to the process of colony multiplication (Ruttner 1957). In other words the mating season in honeybees is inevitably linked to the swarming season. Reproductive swarming depends on favorable environmental conditions. Specifically, ample pollen and nectar must be available for two reasons: to produce enough bees before colony fission and to support the swarms which do not have combs or any honey storage at the beginning(Seeley 1985). For survival, a new swarm needs more or less immediate access to nectar and pollen for comb building and brood rearing. Otherwise the natural mortality of worker cannot be compensated and eventually the swarm (the new colony) dwindles beyond a critical threshold. Therefore the mating season in honeybee populations depends on seasonal blooming cycles. This holds true for allopatric *Apis mellifera* in Africa and Europe (Ruttner 1992) and for populations of sympatric Asian species. Accordingly, in Sri Lanka (Koeniger and Wijayagunasekera 1976), in Thailand (Rinderer et al.1993) and in Borneo (Koeniger et al.1996) all sympatric *Apis* produced drones simultaneously. We assume, because of the uniform mode of colony multiplication by swarming within the genus, that there is not much "evolutionary flexibility" to change the reproductive season between sympatric honeybee species.

Mating locations

The drones of *Apis* species do not mate within their colony when they would only meet young sister queens. By leaving the hive, drones avoid inbreeding. For discussing the genus *Apis* we start with a short description and review of the drone congregation areas of honeybee species.

During mating flights, *Apis mellifera* drones congregate in open air above their drone congregation area (Zimarlicki and Morse 1963) where they remain flying in wide loops until they return to the colony to feed (Ruttner and Ruttner 1965). Congregation areas have usually a diameter of 30 - 200m. More recently, the area above which the drones flew was measured by radar as 1600m^2 (Loper *et al*.1987). Nonetheless, a congregation area has a limited spatial extension and *Apis mellifera drones* are not attracted by a queen flying outside the area (Ruttner and Ruttner 1965). Depending on weather conditions *Apis mellifera* drone populations fly at a preferential height above the ground that varies from 5 - 40m above the ground.

Since virgin queens commence their mating flights significantly later than drones, the congregation of drones is formed irrespective of the presence of a queen. The same drone congregation area was visited by *Apis mellifera* drones each season for more than 30 years (Koeniger et al.1989, Pechhacker unpublished data). Near Selbourne (England) a drone congregation area has been known for 197 years (Tribe 1982). In *Apis mellifera*, several drone congregations are found within the flight range of a colony (Zimarlicki and Morse1963,

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Ruttner and Ruttner 1966, Ruttner and Ruttner 1972). Drones of *Apis mellifera* were found visiting a drone congregation area at a distance up to 8 km from their colonies and drones from a considerable number of different colonies and apiaries were found on drone congregation areas (Ruttner and Ruttner 1965). Calculations of the relatedness of drones captures in a congregation area in Germany revealed that these drones originated from about 240 different colonies and *Apis mellifera* probably represents one of the most elaborate panmictic systems possible among terrestrial animals (Baudry *et al*. 1998). The physiographical structure of *Apis mellifera* drone congregation areas seems to vary greatly (Ruttner and Ruttner 1966). In plains and less structured areas, however, drones are reported to be distributed more uniformly and were attracted to queens where ever they were placed (Tribe 1982, Lahner 1998, Butler *et al*.1964). The local conditions that make the *Apis mellifera* drones to stay or return to a drone congregation area remained unknown (Koeniger *et al*.1988, Ruttner and Ruttner 1966).

In contrast to *Apis mellifera* drones which congregate in open air, *Apis cerana indica* drones in Sri Lanka and in Borneo accumulate in close proximity to trees. These drones restrict their flight to an open space within or near to canopies of trees. They do not follow a (caged) queen far into open space above or at the side of the canopy (Punchihewa *et al*.1990, Koeniger *et al.*1998). The distance between the drone congregation area and the drone's colonies is clearly smaller than in *Apis mellifera* and ranges up to 2 km (Tingek unpublished data). In Japan, however, drones of *Apis cerana japonica* congregate in the open air high above prominent trees (Yoshida *et al*.1993, Fujiwara *et al*.1994). In Germany, *Apis cerana indica* drones originating from Northern Pakistan visited a drone congregation area in an open valley far away from trees (Ruttner 1973, Ruttner *et al.*1972). All together drone congregation areas of *Apis cerana* show degree of variability since these differences can occur within the same subspecies (*Apis cerana indica*). The specific features of those drone congregation area maybe mainly a result of local adaptations to environmental factors. For example, avoiding predators like birds (*Merops spec.* etc.) By flying near or within a tree's canopy might have a higher selective advantage under tropical conditions than in mountains of Northern Pakistan (Punchihewa 1990). Considering the limited data available(in comparison to *Apis mellifera*), we expect that even a wider range of differences among the drone congregation areas of *Apis cerana* may become apparent with further research on Asian honeybees.

Drone congregations of *Apis koschevnikovi* were regularly observed to occur under thick cover of vegetation and the height above the ground of different drone congregation areas varied between 1.5 to 12m (Koeniger *et al*. 1998). At present, there is no information available on the drone congregation areas of the other 2 cavity dwelling honeybees *Apis nuluensis* and *Apis nigrocincta.*

In Borneo, drones of *Apis dorsata* congregate under the canopy of tall emergent trees. The eminent tall tree tops seem to serve as a visual landmark and applying that criterion several 'new' *Apis dorsata* drone congregation areas were located (Koeniger *et al*.1988). Recently, several *Apis dorsata* drone congregation areas were detected under tall trees in Sri Lanka (Punchihewa unpublished data). The drones of *Apis dorsata* assemble under the umbrella of the canopy and do not follow queens which are moved into the open air. Further, drone attraction showed a maximum 3-5 meters below the canopy. The height above the ground ranged between 10 and 35m depending on the size of the tree (Koeniger *et al*.1988). The drone congregation area of the other giant honeybee

species *Apis laboriosa* remains undiscovered. Also the drone congregation areas of the dwarf honeybees *Apis andrenifor is* and *Apis florea* are not yet found.

It is not surprising that among allopatric species, drone congregation areas show similarities. Some convincing evidence for this similarity came from Ruttner (Ruttner 1973). In Germany drones from imported *Apis cerana indica* colonies (which originated from Pakistan) were caught together with simultaneous flying *Apis mellifera carnica* at the same drone congregation area. An estimation of drone numbers in the nearly colonies (*Apis cerana* and *Apis mellifera*) showed that the ratio of *Apis cerana* drones at that drone congregation area was similar to that of *Apis mellifera* drones. A second case of heterospecific drone mixing, although to a much lesser extent, was recorded from drone congregation areas in Japan. In that study, a small number of heterospecific drones were caught at each congregation area (Yoshida *et al*.1993).

At present, the only comparative observations and experiments on drone congregation areas of sympatric Asian honeybees have been carried out in Borneo (Tenom). So we must restrict the following section to the situation in Tenom. Further, we caution that conclusions remain more or less preliminary until they are confirmed by investigations from other places. During our experiments, fairly large numbers of honeybee colonies from 4 species were found. *Apis cerana indica* and *Apis kochevnikovi* colonies were kept in modern hives and both species supplemented a larger natural population of feral colonies. About 50 to 100 *Apis dorsata* colonies were found nesting in several bee trees near (within a radius of 5 km) the experimental area. *Apis andreniformis* foragers were frequently observed on various flowers. During our study period of 10 years, we located 5-10 colonies of this species each season.

Because of the small size and the hidden nest sites, we assume that this was only a rather small part of a sizeable population of *Apis andreniformis* in the area. However, drone congregation areas of *Apis andreniformis* were not detected.

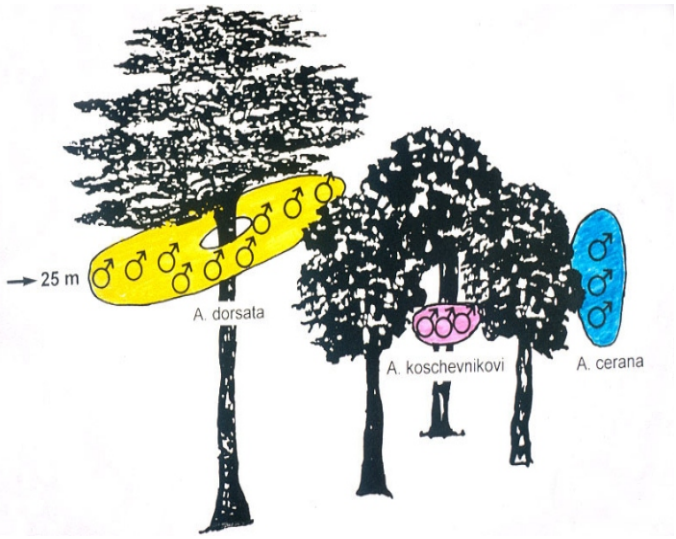

Fig. 1: Drone congregation areas in Sabah (Borneo).*A. dorsata* drones congregated directly under the canopy of high emergent trees. *A. koschevnikovi* drones congregated under the thick cover of branches and trees. *A. cerana* drones remained near the branches of neighbouring vegetation. All three drone congregation areas were within a distance of 30 m. ♂ Spaces occupied by drone flight.

A general scheme (Fig. 1) is mainly based on observations of 3 different drone congregation areas (Koeniger *et al*.1989). At these places the major landmark was an outstanding tree top which clearly protruded from the horizon line. Drones of *Apis cerana indica* had their maximal flight frequency measured by attraction to our standard dummies (Fig. 2) slightly outside the canopy of the trees and larger shrubs about 10 to 12m above the ground. When disturbed by birds or by our insect net, *Apis cerana* drones escaped rapidly into the cover of the branches. Drones of *Apis koschevnikovi* remained under the

dense cover of the canopy and flew into a space 6 to 8m above the ground. *Apis dorsata* drones were flying under the first layer of branches in a height of 20 to 25m. So the distribution of drones resulted in a clear spatial separation without any overlap between the species.

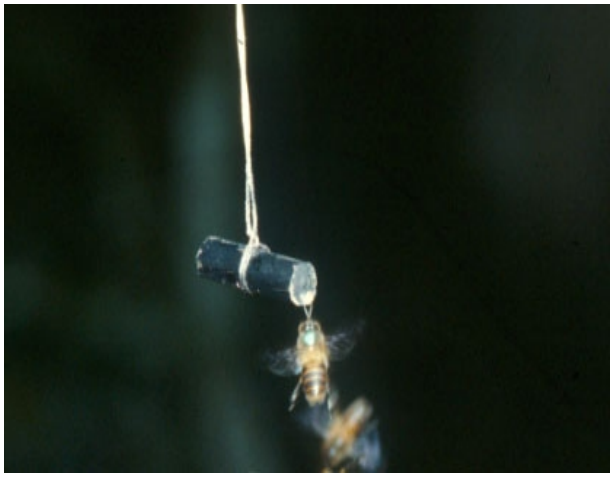

Fig. 2.: *A. koschevnikovi* drone attracted to the standard dummy. A section of black pencil (length 34 mm, diameter 8 mm) was tied to a thread and impregnated with 1 mg 9-ODA.

Sexual signals

The highly developed social life of honeybees has affected the mating behaviour in several ways. The sex ratio is strongly male biased, colonies produce some hundred times more drones than queens (Ruttner 1957). Taking into account that *Apis* drones perform multiple nuptial flights while a queen flies only once or twice for mating, the effective male bias at a drone congregation area is further increased. As a consequence male-male competition (Thornhill and Alcock 1983) must have a major impact on drone behaviour. In pursuit of a flying virgin queen the speed (time) of a drone to reach his target will gain the highest priority. Under these conditions drones will depend on simple and fast detectable stimuli for queen recognition. Further, to win this race, an immediate reaction to a first, albeit uncertain signal of a virgin queen will be a better choice, because the probability for the drone to encounter a second queen is close to Zero (Gries and Koeniger 1996).

The first reaction of drones to visual stimuli seems to depend on "unspecific" movements. At a congregation area, drones of *Apis mellifera* react to various moving objects by a fast and short turning reaction. Flying birds, butterflies and even stones thrown into the air area will momentarily attract some drone (Jean-Prost 1957). Without the presence of pheromones, however, these objects will never release any pursue or a consistent attraction. According to Strang (Strang 1970) the optimal surface of a queen dummy (impregnated with pheromone: 5 mg 9-ODA) was slightly larger than a queen (about $3cm^2$). With further increasing size the attraction to drones decreased. Black (and red) was the optimal colour. In other words, the visual signal attracting *Apis mellifera* drones seems to be rather unspecific: fast moving, dark objects of a size slightly larger than a queen seem to be nearly optimal.

The major active chemical component of the *Apis mellifera* queen's mandibular gland was identified as (E)-9-oxo-2-decenoic acid (9-ODA) by Callow and Johnston (1960). Among several other important biological functions, 9-ODA was found to be the main component of the *Apis mellifera* queen's sex attractant (Gary 1962, Pain and Ruttner 1963). Later, it was demonstrated that extracts of queens of 3 other *Apis* species (*Apis florea*, *Apis cerana* and *Apis dorsata*) attract *Apis mellifera* drones and that these extracts contained 9-ODA. *Apis dorsata* and *Apis cerana* queens had a quantity(150 to 300

mg) of 9-ODA similar to that of the *Apis mellifera* queen (Butler *et al.*1967, Shearer *et al.*1970).

Consequently, dead, extracted queens or black dummies of similar size (Fig. 2) impregnated with 1mg of 9-ODA successfully attracted drones of *Apis cerana* (Ruttner 1973, Punchihewa *et al.*1990, Yoshida *et al.* 1993) *Apis dorsata* (Koeniger *et al*.1988) and *Apis koschevnikovi*. Underlining the uniform mechanism of drone attraction further, Gries (1997) attracted drones of *Apis dorsata*, *Apis mellifer*a, *Apis cerana* and *Apis koschevnikov*i to the same dummy. Drones were not only attracted but also started to grasp the dummy and to initiate copulation. However, for successful copulation an opening at the end of the dummy (like an open sting chamber) is required (Fig. 3) (Gary and Marston 1971).

Fig. 3.: Copulation dummy with two *A. koschevnikovi* drones. At both ends of the standard dummy (1 mg 9-ODA) the hollowed abdomen of an *Apis* queen was glued. The *A. koschevnikovi* drones were attracted, copulated and became stuck in the abdomen.

Plettner *et al.* (1997) found specific differences in the mandibular gland signals between queens of *Apis mellifera*, *Apis dorsata*, *Apis flore*a and *Apis andreniformis*. The question must be addressed whether or not the results of the behavioural experiments explore the natural situation. We can not exclude that the demonstrated interspecific Drone attraction was caused by our experimental techniques: for example excessive use of 9-ODA or any other super stimuli of the dummies.

Apparently, the drastic male bias at the drone congregation area and the resulting competition among drones has led to a fast, simple and uniform mechanism of queen recognition. The main olfactory signal and essential sex pheromone seems to be 9-ODA in all Apis species. The specific differences in the queen's pheromone spectrum among the species (Plettner *et al*. 1997) may result in a reduced attraction of the heterospecific queen.

It will however not prevent interspecific copulations as Ruttner and Maul (1983) demonstrated. That leads to the conclusion that the differences of sexual signals do not play a major role as behavioural barrier between sympatric honeybee species.

Different daily mating periods

The "allopatric situation"

As discussed before, the natural distribution of *Apis mellifera* generally does not overlap with the distribution of other *Apis species* (Ruttner 1988). Thus for *Apis mellifera*, we can assume that an evolution "under the condition of being the only honeybee species at a place" has shaped the mating behaviour and the daily mating period. Drones of European races of *Apis mellifera* start flying shortly after the sun passes the zenith (12.15 hrs.) and stop in the late afternoon (17.00 hrs.) (Ruttner 1966). In Germany a comparison of

drone flight times between *Apis mellifera ligustica* and *Apis mellifera carnica* showed no significant differences (Drescher 1969, Koeniger *et al.* 1989). Observations in Africa, near Pretoria, with *Apis mellifera scutellata* resulted in period from 12.45 to 16.45 hrs. (Tribe 1982) and more recently Lahner (1998) reported with *Apis mellifera monticola* drone flight activity in Malawi to occur from 11.20 to 16.00 hrs.

Apis mellifera queens perform their mating flights during the peak of drone flight. Successful mating flights of queens (returning with mating sign!) occurred in Austria between 14.20 and 16.10 hrs. (Koeniger *et al.* 1989). In Africa *Apis mellifera monticola* mated queens returned between 13.00 to 15.30 hrs. (Lahner 1998).

Overall, the mating flight period of *Apis mellifera* invariably starts shortly afternoon and stretches over a period of 4 to 5 hours. The differences reported so far were within a short range and did not exceed 1 hour. The period of actual mating (as documented by queens returning with mating sign) is considerably shorter (2 to 3 hours). Queens fly later and stop their flight activity earlier than drones. Compared to other behavioural characters (defence, swarming etc.) The daily mating period of *Apis mellifera* seems to be rather uniform in Africa and Europe.

Among the Asian honeybee species *Apis cerana* has the most extended natural distribution. In consequence it overlaps with many of the other Asian *Apis* species (Ruttner 1988). Regionally, there are however large areas where *Apis cerana* is the only honeybee. Within the Asian continent these are mainly in the northern part of their range, in mountain ranges and in the Japanese islands (with the exception of Hokkaido).

A daily *Apis cerana* mating period from 12.30 to 16.00 hrs. was reported from Bihar in North India. Drones of *Apis cerana indica* (originating from the mountains of the North West Frontier Province of Pakistan) flew between 12.00 to 15.30 hrs. in Germany (Ruttner *et al.* 1972). Verma (1991) observed mating Flights of *Apis cerana indica* queens in the Shimla Hills (North India) between 12.30 and 15.30 hrs. In Japan, drones of *Apis cerana japonica* flew from 13.15 to 17.00 hrs. and successful mating flights of *Apis cerana japonica* queens occurred between 14.35 and 16.35 hrs. (Yoshida *et al*. 1994). The mating period of *Apis mellifera* and the observations from regions where *Apis cerana* occurs as the only Apis species show a striking degree of similarity. The overall duration of drone's and queen's flights and the timing during the early afternoon seems to be nearly identical in *Apis Mellifera* and 'allopatric' populations of *Apis cerana.*

The "sympatric situation"

The Table 1 will focus on observations and research which present data on mating flights of sympatric species at one location.

Apis andreniformis drones (Table 1) have a uniform short flight period after 12.00 hrs. The period of *Apis andreniformis* queen flights was between 12.33 to 12.50 hrs. in Sabah (Koeniger *et al*. 2000).

Apis florea drones (Table 2) show remarkable differences. In Sri Lanka drones flew earlier than South East Thailand. According to Koeniger et al (1989) drone flight period of *Apis florea* in Bangkok was be between 13.45 and 15.30 hrs.and ended more than 1 hours earlier compared to the above data (Table 2). Also successful mating flights of *Apis florea* queens in Bangkok were observed between 14.04 and 14.25 hrs. Apparently, some variability of *Apis florea* mating periods occurs within Thailand. Perhaps, the earlier mating period (in comparison to Table 2) is typical for regions (like Bangkok) where *Apis florea* is the only

dwarf bee species.

Apis cerana exhibits more variability in drone flight period than other *Apis s*pecies.

Koeniger and Vijayagunasekera (1976) discussed that *Apis florea* in Sri Lanka occupies the time window which is nearest to

Table 1 Drone flight periods of sympatric Asian honeybee species

1st author (year) Locality	Koeniger (1976) Sri Lanka (hrs)	Rinderer (1993) Thailand (hrs.)	Koeniger (1996) Sabah (hrs.)
A.andreniformis		12.15 to 13.45	12.00 to 13.45
A.florea	12.00 to 14.30	14.00 to 16.45	
A.cerana	16.15 to 17.15	15.15 to 17.30	14.00 - 15.30
A.koschevnikovi			16.45 - 18.30
A.dorsata	18.00 to 18.45	18.15 to 18.45	18.15 - 19.05

Table 2 Number of spermatozoa of one drone reaching the spermatheca (million)

Species	spermatozoa in spermatheca (n queens)	spermatoz. in ves.sem.	mean paternity	effective paternity	n spermat. per drone in spermatheca	% spermatozoa of one drone reaching the spermatheca
A. a	0.78 (7)	0.13	13.5	9.1	0.086	66%
A. f	1.05 (15)	0.43	8.0	5.6	0.187	44%
A. c	1.35 (12)	1.1	18	12	0.113	10%
A. k	2.13 (4)	1.7	16.3	10.5	0.203	12%
A. m	4.73 (126)	12.7	13.8	12.4	0.370	03%
A. d	3.94 (8)	2.46	22.4	22.8	0.173	07%

The drone flight period of *Apis cerana indica* drones (Table 1) in Sri Lanka was confirmed by Punchihewa *et al.* (1990). Accordingly, queens successfully mated between 16.15 to 16.55 hrs.(Punchihewa *et al*. 1990, Punchihewa 1990). The mating period of *Apis cerana indica* in Sri Lanka is the latest so far recorded for this species. *Apis koschevnikovi* drones fly during a long period of more than 2 hours. Queens flew between 17.00 and 18.15 hrs. (Koeniger *et al*. 1994). *Apis dorsata* fly consistently at sunset. The flight period of *Apis dorsata* drones is very short. Drones of this species perform daily only a single flight (Koeniger *et al*. 1988). In Borneo a slight overlap with *Apis koschevnikovi* drones occurred. This was, however, too slight to effect the reproductive isolation.

the 'allopatric' mating period. Therefore, theyargued *Apis florea* was the original *Apis* species to arrive at the island of Sri Lanka. Consequently the species with later periods would have reached Sri Lanka some time later. With the evidence (Table.2) available today, however, a general and uniform pattern related to taxonomy became apparent: The first position directly afternoon is held by a dwarf bee species (*Apis andreniformis* and/or *Apis florea*). The next time window seems to belong to 1 or even 2 cavity dwelling species (*Apis cerana* and *Apis koschevnikov*i) and at the very end of the day, just around sunset, *Apis dorsata* holds its mating time. It seems to be unlikely, that the similarity in this sequence of mating periods has originated by chance in 3 different locations (Sri Lanka, Thailand and Sabah).

Another possibility, that this sequence has evolved once in South East Asia and spread unchanged to the above places, seems to be equally unlikely. Therefore we hypothesise that this pattern originated from a (yet unknown) mechanism of interspecific reproductive competition, which causes predictable results independently of place and environment. Without exception, the temporal sequence of mating periods is strictly correlated with the size, it starts with the smallest *Apis* species (*Apis andreniformis)* and ends with the largest sympatric species (*Apis dorsata*).

It is rather tempting to speculate how drone behaviour can result in this sequence: The basic reaction of *Apis* drones is directed to queens which are larger than fellow drones. So smaller drones may try to copulate unidirectional with larger drones, excluding them from access to queens. However, these speculations are premature and the question whether the size had a direct effect or rather size correlated factors caused the above sequence remains unsolved.

Our above hypothesis however has come to a large scale test. Recently, *Apis florea* was involuntarily introduced to Africa (Mogga and Ruttner 1988). Fairly large populations established in the region of Khartoum (where no feral *Apis mellifera* live) and *Apis florea* colonies are spreading to South, along the Nile (Mogga 1994). Eventually *Apis florea* will reach the habitat of *Apis mellifera scutellata*. In the likely event of a sympatric co-existence we predict that as a result of a fast natural selection process *Apis florea* drones will fly prior to *Apis mellifera* drones.

Whenever the sequence of separated mating periods was established, it facilitated sympatric co-existence and *Apis* species could spread simultaneously sharing their habitats. However, in the case where they spread into a 'new' territory alone, each species will shift towards the 'allopatric' mating period. Several 'allopatric' behaviour of bees may serve as an example for the latter phenomenon: The allopatric population of *Apis cerana* from Northern India and Pakistan were already discussed above. Further, the open nesting giant honeybee species *Apis laboriosa* adapted to high altitudes of the Himalayas has a drone flight period between 12.20 and 14.20 hrs. (Underwood 1990). Likewise, *Apis nuluensis* is the only honeybee species in the mountains of Borneo above 1700 m and its drone flight period is between 10.45 to 13.15 hrs. No observations on queen flights are yet available and as the drone flight was recorded from 1 colony only (this applies to *Apis laboriosa* too), there is a need of additional confirmation.

Arguably, the time sharing mating pattern of sympatric *Apis* species has evolved to a nearly perfect behavioural barrier. In consideration of the temporal pattern of the *Apis* mating behaviour a separate daily mating period becomes operational earlier than several complicated and "risky' events in a behavioural sequence. After this successful "a priori" reproductive isolation was established, previously functional mechanisms which operated on later steps in mating behaviour became less meaningful. In other words flying during their daily mating period drones and queens of any *Apis* species do not gain further by maintaining different sexual signals or species specific mating places.

What is involved in timing of mating flights? Taber (1964) confined an *Apis mellifera* colony in a cool dark room for 12 hours inducing earlier drone flight on the following day. Yoshida and Yamazaki (1993) were able to shift the flight period of *Apis mellifera* drones by changing the photo period. The above results suggest that the drone flight period seems to depend on an internal clock. Koeniger *et al.* 1994 used cross faster techniques. They introduced drones of *Apis koschevnikovi* and *Apis cerana* into alien colonies. As a result, *Apis koschevnikovi*

drones flew during their species specific mating period independent from their *Apis cerana* host colony. Similarly, *Apis cerana* drones followed their own mating period andnot the *Apis koschevnikovi* colony's time table. Later, virgin *Apis koschevnikovi* queen were introduced into *Apis cerana* colonies and flew at their own species mating period (Koeniger *et al.* 1996). Drones and queens seem to decide on the right time for mating on the basis of an 'inherited time table'. Consequently, a direct evolutionary impact on the individual drone or queen and selection for changes in mating period becomes operational and may act faster than any effect via the colony (workers). Thus fast adaptations to predatory pressure, other environmental alterations or even to "new" honeybee species are facilitated.

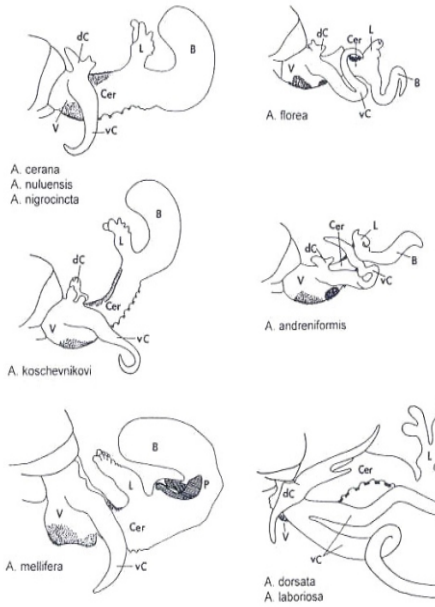

Figure 4. Everted endophalli of nine *Apis* species (lateral view). Symbols for all figures: B: bulbus; Cer: cervix; P: chitinous plates of bulbus; dC: dorsal cornus; L: lobe; V: vestibulum; vC: ventral cornua. ▨: hairy fields.

Copulation

The queen's genital tracts within the genus *Apis* are similar and simple in principle. The genital chamber opens at the base of the sting. Its outer part is the bursa copulatrix, the inner part the vagina with the valvula vaginalis. Small differences concerning the valvula vaginalis were reported by Camargo (1972).

In comparison, differences of male genitalia between honeybee species are very impressive. Generally, in *Apis* the copulative organ is a membranous endophallus which is differentiated into a broad vestibulum with its cornua, the slender cervix and the thick bulb with its lobe. In situ they look quite similar. The characteristic marks to distinguish them seem to be limited to differences in the hairy fields, in the form of the cornua and the lobe (Koeniger *et al.* 1991, Patinawin and Wongsiri 1993). During copulation the endophallus is everted successively and introduced into the queen. The differences of the everted endophalli in form and size become striking (Fig.4). For example the cornua bend either dorsally or ventrally in the everted endophallus, the same is true for the bulbus (Koeniger *et al.* 1991). These differences are expected to have major functional consequences in case of copulation between species belonging to different taxonomic groups (hive dwelling, the giant and the dwarf honeybees).

However, within these groups there are many similarities. In all dwelling species it seems to be mainly the large endophallus which connects the drone to the flying queen. Filled under high muscular pressure with mucus of the male accessory glands and air it seems to guarantee a sufficiently strong connection between the flying queen and the paralysed drone until sperm transfer into the oviducts is completed (Koeniger and Koeniger 1991). The short thick cornua with its "orange coloured" sticky and greasy secretion may contribute to strengthen the attachment and later, after separation of the pair, it sticks to the mating sign and keeps it in place The giant honey bees have a more elongated endophallus with four long curled

cornua also covered by an "orange coloured" sticky secretion. The elongation of the Endophallus is mainly caused by the extended cervix (Fig. 4). The drones also get paralysed during copulation. In these bees the broadened metatarsi seem to reinforce the attachment to the queen (Ruttner 1975).

The everted endophallus of the dwarf honey bees shows many differences to the previous ones. The so called bulbus is a thin tube (Fig. 4) and the mucus glands are tiny. They cannot produce enough mucus to strengthen the connection between thecopulating pair. Instead they have a forceps- like appendix at the metatarsus of the hind leg which is a bit shorter in Apis andreniformis than in Apis florea. With these "thumbs" the drone locks himself to the hind legs of the queen (Ruttner 1988), supported again by the sticky cornua pressed into the sting chamber. Thus the pair stays connected until the queen turns her legs in a way that the drone is released. This different mode of attachment seems to have evolved together with a changed form of the bulbus: it ends in a fine tip enables the drone to deposit the sperm into the thin spermaduct instead of the wide oviducts.

Mating sign

After copulation, mating signs are left in the sting chamber of the *Apis* queen. However, the queen of all *Apis* species mates several times during a single mating flight. So the mating sign does not prevent further mating but it must be removed by the next drone.

Mating signs were described in the cavity dwelling species (Woyke 1960, Woyke 1975 Koeniger et al. 1994). They consist of mucus from the male accessory glands, secretions of the bulbus gland and orange coloured secretions of the cornual glands (Koeniger *et al.* 1996, Koeniger and Hänel 1996). In *Apis mellifera* there are also chitin plates from the bulbus. In *Apis andreniformis* only the secretion of the cornual gland was found in the sting chamber (Koeniger *et al.* 2000). While mating signs look similar in the Asian species *Apis cerana* and *Apis koschevnikovi,* it is clearly different in *Apis mellifera.*

In *Apis mellifera* it could be observed that drones are able to remove the mating sign at the beginning of copulation. It is attached to the hairy field on the ventral side at the basis of the endophallus. At the end of mating every drone leaves its own sign (Trjasko 1957, Koeniger 1986). After the last copulation more than 70% mated queens return carrying the last mating sign in her sting chamber. After return from the mating flight it is removed mostly by the queen rubbing the abdomen on the comb. As queens of *Apis cerana* and *Apis koschevnikovi* also return from one mating flight with a mating sign and their oviducts filled with sperm of about 5 to 10 drones (Woyke 1975, Koeniger *et al.* 1994), drones of these species, too, are able to remove the mating sign of their predecessors.

In *Apis dorsata* queens return from mating flights after sun set during darkness. No mating signs were noticed protruding from the sting chamber and the queen was permitted to enter back into the colony (Tan *et al.* 1999). On the other hand *Apis dorsata* drones have well developed mucus glands (own observations) and in a video film on mating with dummies by Gries (unpublished Results) the deposition of a white plug on the dummy was demonstrated.

Apis florea and *Apis andreniformis* have tiny mucus glands. While in *Apis florea* no section of drones were found in the sting chamber of queens returning from mating flights (Koeniger *et al*. 1989), all 3 observed queens of *Apis andreniformis* had the reddish yellow cornual secretion protruding from the tip of the abdomen (Koeniger *et al*. 2000).

Sperm transfer

The percentage of a drone's spermatozoa stored in the spermatheca is quite different in different species, although DNA studies

Table 3 Queens of 4 species instrumentally inseminated with sperm of various Heterospecific species

Sperm	*mellifera*	*cerana*	*koschev.*	*dorsata*
Queens				
mellifera		+(1.2)		
cerana	+(3)			+(4)
koschevnikovi		+(5)		+(2)
florea	+(7)			

( ) n queens,

revealed the number of effective matings are similar among the species (Estoup *et al.* 1994, Moritz *et al.* 1995), Rinderer *et al.* 1998, Oldroyd *et al.* 1995, Oldroyd *et al*. 1996, Oldroyd et al. 1997, Oldroyd *et al.* 1998). The total number of spermatozoa in the queen's spermatheca divided by the effective number of matings indicates the amount of spermatozoa contributes by each drone (table 2). For example, an *Apis mellifera* drone produces about 12.7 million spermatozoa, but only about 370,000 reach the spermatheca. Though it is the highest number of all species it corresponds to only about 3% of the spermatozoa of a single drone. This number is around 10% in *Apis koschevnikovi*, *Apis cerana* and *Apis dorsata*. The other extreme occurs in *Apis andreniformis*: 1 drone produces 0.13 million sperms of which an average of 66% in average are present in the spermatheca (Koeniger *et al.* 1990). These calculations differ slightly to those of Oldroyd *et al.* 1998 and Palmer and Oldroyd (2001), but do not change our conclusions.

These findings support the idea that the mode of sperm transfer (sperm injection into oviducts versus sperm injection into spermaduct) influences the filling process of the spermatheca. Injection into the oviducts results in a waste of more than 90% spermatozoa, whereas with injection in the spermaduct (Koeniger *et al*. 2000) only about half is rejected. However, the sample size in the Asian species remains too small to state this as a common rule.

Sperm storage

The technique of instrumental insemination permitted the study of heterospecific sperm transfer and storage. The following combinations have been done (Table 3).

In all cases some semen reached the spermatheca. In the interspecific and intraspecific inseminations of an *Apis cerana* queen "twice as many *mellifera* spermatozoa reached the spermatheca when injected into the oviducts than in case of *cerana* spermatozoa. Thus heterospecific insemination is as efficient as homospecific". Up to 1.9 million living spermatozoa were counted in the spermatheca of *Apis cerana* (Ruttner and Maul 1983).

In the interspecific and intraspecific inseminations of *Apis koschevnikovi* and *Apis cerana* about the same percentage of spermatozoa (8-9%) reached the spermatheca (Table 2), independent of hetero-or conspecific sperm. The percentage corresponds to that after natural mating (Table 2). In all cases spermatozoa in the spermatheca were viable when queens were dissected 3 to 40 days after insemination. The Amount of spermatozoa was below 1 million, except in *Apis koschevnikovi* queens inseminated with conspecific sperm.

After insemination of *Apis koschevnikovi* with *Apis dorsata* sperm the percentage of spermatzoa reaching spermatheca was quite low. But with 2 experiments only and no reciprocal insemination these results must be considered preliminary. Woyke (1993) reports that after inseminating *Apis florea* queens instrumentally with *Apis mellifera* sperm

some spermatozoa entered the spermatheca but he did not report the percentage. To a certain extent a comparable migration of conspecific spermatozoa was demonstrated in *Apis mellifera* queens (Gessner and Ruttner 1977). No reciprocal inseminations were done. These data suggest that the physiology of the genital duct and its fluid is similar throughout all species.

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