

## PHEROMONES AND REPRODUCTION IN BRAZILIAN STINGLESS BEES

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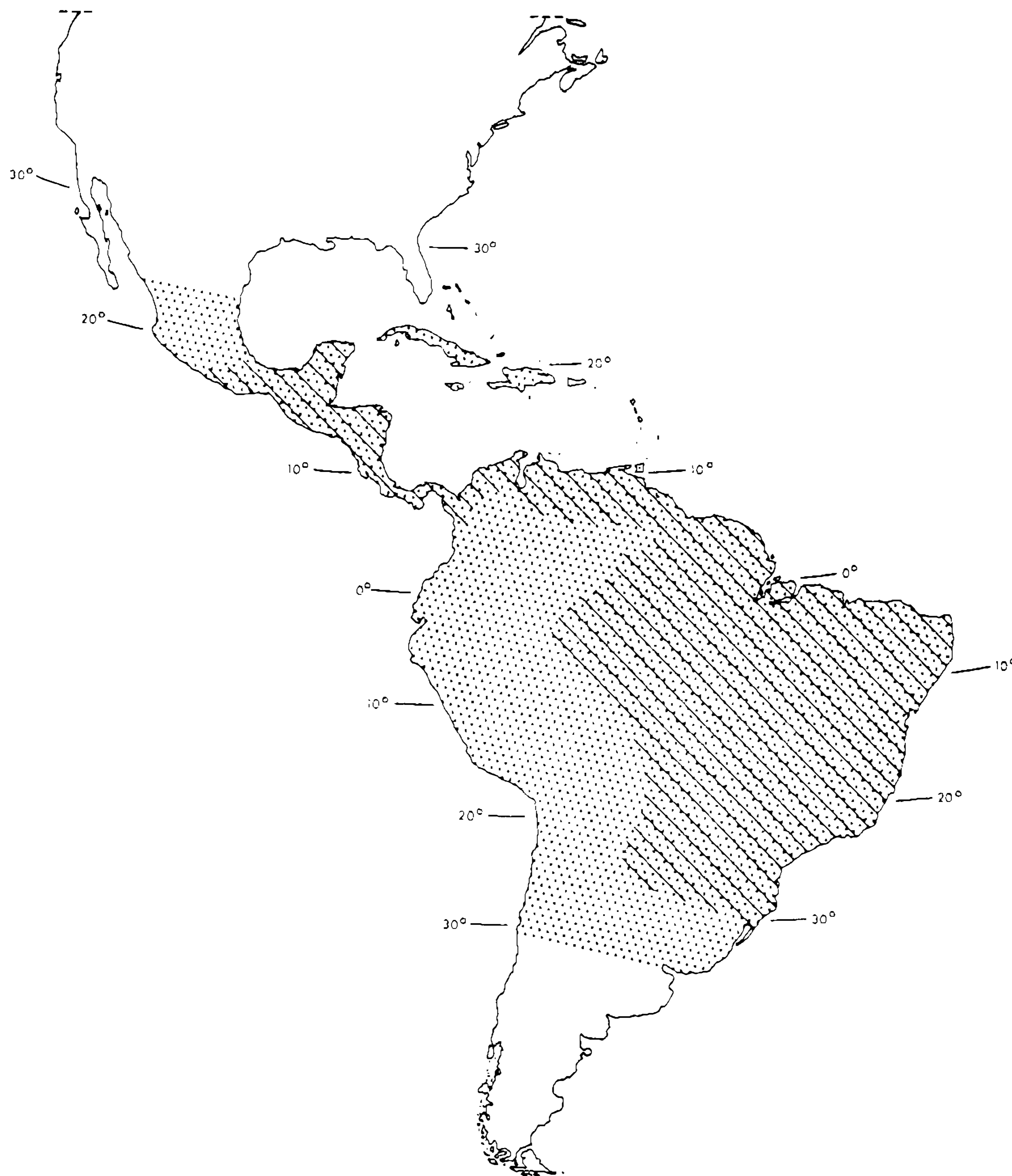


Fig. 1: Biogeographic distribution of stingless bees in the neotropics. Hatched area indicates the occurrence of the superspecies *Scaptotrigona postica*. There are no stingless bees in high altitudes of the Andes and in the deserts.

Stingless bees or Meliponids are of pan-tropical distribution. Most of the approximately 500 recent species, about 300 or more, are living in the neotropics ranging from Mexico to Argentina (Fig. 1). The stingless bees have achieved a highly eusocial level of colonial organization similar to the honey bees. Nearly all species are monogynous. The nests are mainly built in cavities and are found in the ground, in termite nests or in hollow trees. Normally at most an entrance funnel is visible from outside. Therefore, experiments require a transfer of the nest into the laboratory. Special observation hives have been constructed for different pur-

poses (Sakagami, 1966; Nogueira-Neto, 1970). During the past 15 years we have studied the biology of reproduction in stingless bees by analysing the occurrence of vitellogenin, mating biology and pheromones.

### MATERIALS

Our studies were carried out in cooperation with several Brazilian research groups in the states of São Paulo, Paraná, and Rio Grande do Sul. One of our main subjects is *Scaptotrigona postica*, a common Trigonine species occurring from Central America to Argentina (Fig. 1). We are keeping a number of colonies of the subspecies *Scaptotrigona postica depilis* in a meliponary on the USP campus at Ribeirão Preto



Fig. 2: Brood nest of *Scaptotrigona postica* surrounded by irregular membranes of involucrum. Notice the horizontal layers of brood combs. New cells are always added at the margin. Open brood cells with a narrowed rim are ready for provisioning by the nurse workers and subsequent ovipositioning by the queen. Immediately after an egg has been laid on top of the food sap, the cell is closed by a worker. The larva hatching 3 days later feeds on the provisions. There is no direct contact between larvae and adult bees. (Magnification 1.4 x).



Fig. 3: Close-up shot of an old physogastric queen of *Scaptotrigona postica* surrounded by worker bees mostly of the nurse age. (The open brood cells have been dismantled by the workers after the colony had been disturbed during taking picture. Magnification 2.8 x).

as well as in our laboratory at the University of Tübingen.

METHODS

Caste specific differences in female bee fertility can be quantified using vitellogenin as a parameter (Engels 1974, 1987). Mating biology and interindividual reactions concerning social bee reproduction involve a lot of chemical communication performed by pheromonal messages (Engels 1986; Free, 1987). The identification of volatile compounds found in stingless bees was done by Wittko Francke and coworkers, Dept. of Biochemistry at the University of Hamburg, in the course of a continuous cooperation (cf. Francke 1987; Francke et al., 1987). Bioassays were developed to quantify drone (Engels and Engels 1987) and worker reactions (Engels et al., 1987a). For the latter the uppermost comb can be used as an experimental platform (Fig. 9), as *Scaptotrigona postica* colonies build a brood nest with regular layers of horizontal combs constructed in sequence from bottom to top (Fig. 2).

RESULTS

*The Model: Honey Bee Queen Substance Effects*

Any analysis of pheromones and reproduction in highly eusocial bees has to consider the respective knowledge accumulated on honey bees (Ruttner, 1985, 1987; Velthuis, 1985; Engels, 1987; Free, 1987). In *Apis* queens the main component of the female sex pheromone, (E)-9-oxo-2-decenoic acid, has multifunctional effects (Table I). In addition to the original role as a male attracting lure, the queen substance has many influences directed towards the workers. The effects involved are important constituents of colonial homeostasis: Prevention of the workers from building royal cells and rearing queen larvae and inhibition of the reproductive capacities of the workers, thus keeping the rudimentary females of this caste completely unfertile under queenright conditions. Only in queenless colonies some of the workers become capable of laying eggs (Engels, 1974, 1986, 1987; Free, 1987).

*Queen Fertility in Stingless Bees*

In stingless bees the caste dimorphism is even more pronounced than in honey bees (Engels & Engels, 1977). The old egg laying queen is physogastric (Fig. 3) weighing 120-130 mg compared to about 20 mg of body weight in

TABLE I

Multiple functions of the queen substance in the honey bee, *Apis mellifera*: A model for pheromonal effects in social bee reproduction

Court formation, pheromone transfer by licking workers	
Inhibitory functions:	construction of queen cells worker ovary functions worker egg laying
Stimulatory functions:	worker attraction incl. swarm comb building brood rearing
Mating biology:	drone attraction especially over congregation areas
Main compound:	(E)-9-oxo-2-decenoic acid

the workers of *Scaptotrigona postica*. The ovaries of a newly emerged queen contain only a few previtellogenic egg follicles. No vitellogenin is synthesized (Fig. 4a), and consequently the vitellogenin titer in the hemolymph is on a zero level (Fig. 4b). Therefore, a newborn stingless bee queen is still completely infertile. During the first two or three days of adult life, the young virgin loses about 15% of their body weight (Fig. 4c).

Ten days after emergence, the physiological status of the gyne has changed a lot. Vitellogenin synthesis was initiated and is rapidly increasing in rate (Fig. 4a). By this the hemolymph vitellogenin titer also has attained 15-40% (Fig. 4b). Up to this time the body weight is very low (Fig. 4c). The changes are continued until, at an imaginal age of 15-20 days, a fertile status is achieved by the queen who normally has been mated in the meantime. About 80-90% of the newly synthesized serum protein is vitellogenin (Fig. 4a). Now the vitellogenin titer makes up 50% or more of the soluble hemolymph proteins (Fig. 4b). The body has put on weight according to the development of many vitellogenic egg follicles resulting in a much enlarged ovary. At this moment the queen is capable of laying eggs. Depending on the rate of oviposition, the body weight of an old queen is still increasing until the development of a pronounced physogastric abdomen is finished (Fig. 4c). In queens laying many eggs per day, the vitellogenin titer is lowered somewhat (Fig. 4b) because much yolk precursor material is drained by the numerous vitellogenin incorporating oocytes (Engels, 1973). Only if an old queen is hindered to oviposit is the vitellogenin titer rising again (Fig. 4b). In old stingless bee queens the rate of vitellogenin synthesis remains constantly on a high level (Fig. 4a).

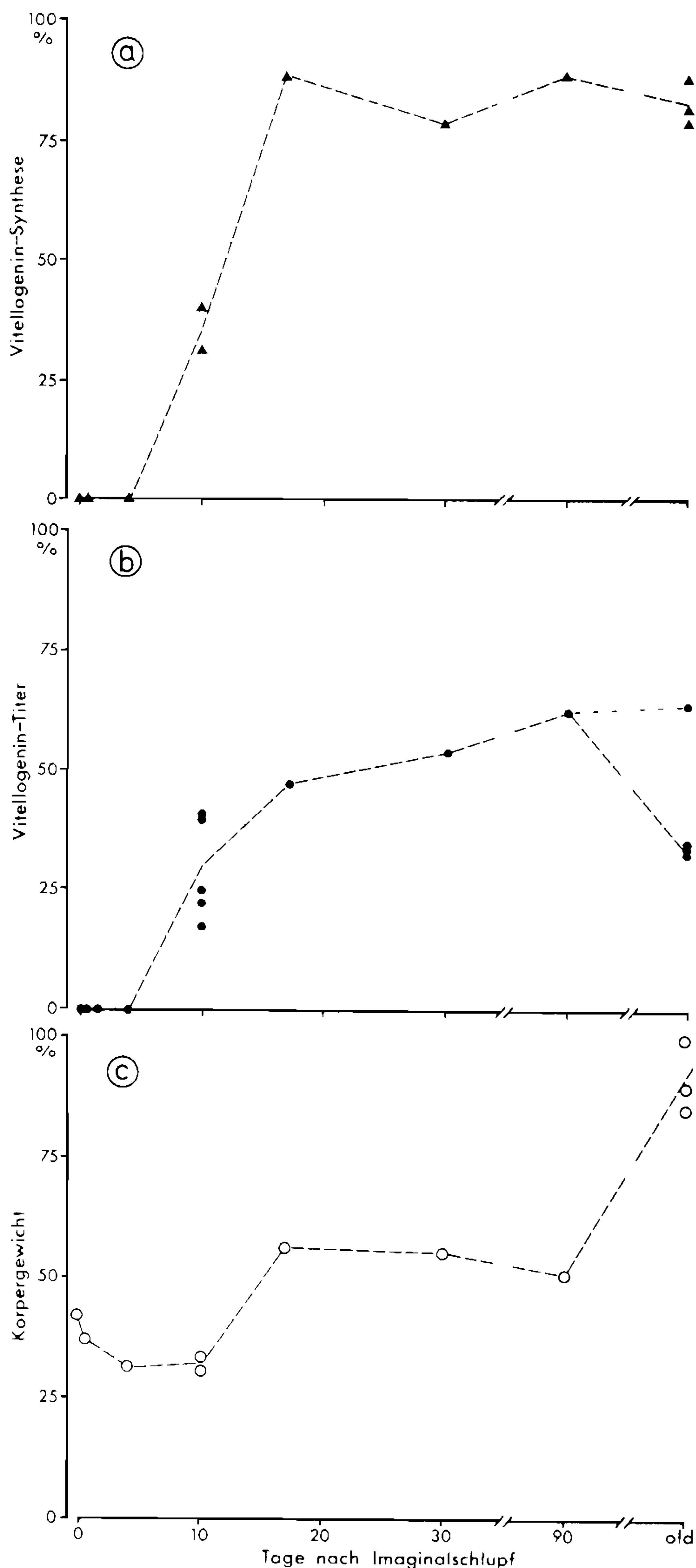


Fig. 4: Changes during the imaginal lifespan of *Scaptotrigona postica* queens until becoming fully fertile and physogastric. a) Portion of vitellogenin from all hemolymph protein synthesis. (Tracer experiments, injection of  $^{14}\text{C}$ -amino acids, 2 h incubation).

b) Relative amount of vitellogenin out of all soluble hemolymph proteins. Distantly broken line = non laying old queens. (Densitoscans of cellogel pherograms).

c) Percentage of the maximal individual body weight of 132 mg recorded in a physogastric egg laying old queen. — All data from individual queens (Engels & Engels, 1977).

#### Worker Fertility in Stingless Bees

In contrast to honey bees, in most stingless bee species the workers are laying eggs also in queenright colonies. In *Scaptotrigona postica*

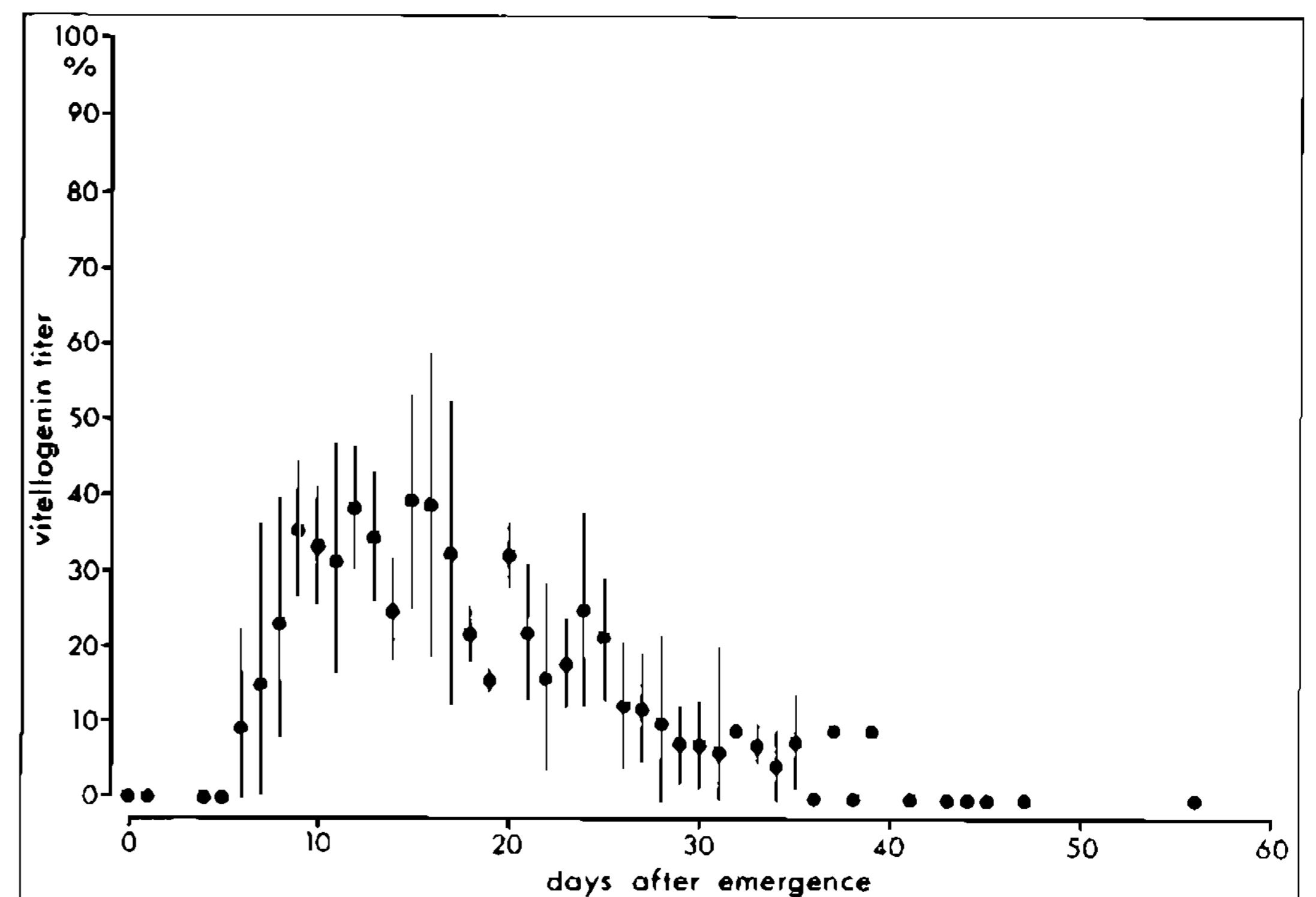


Fig. 5: Changes in relative vitellogenin titer during adult life of queenright workers in *Scaptotrigona postica*. Mean values and standard deviation. (Technique as in Fig. 4b; Engels & Engels, 1977).

the workers mainly produce alimentary eggs which are eaten by the queen immediately before her own oviposition (Akahita et al., 1970). We studied the question whether all the workers become fertile and at which age they are capable of egg laying (Engels & Engels, 1977).

In the newly emerged workers no vitellogenin is found in the hemolymph. From day 5 after emergence vitellogenin is present in increasing amounts (Fig. 5). The maximal titer of 30-40% is observed around day 15. Thereafter a slow decrease occurs. No more vitellogenin is detectable in the hemolymph of foraging workers older than 40 days. With some individual variation such an ontogenetic change in vitellogenin titer is normal to all the worker bees in a queenright colony. Dissection of ovaries proved that only one or two follicles become vitellogenic without any preference of the left or right ovary (cf. Sakagami et al., 1963; Staurengo da Cunha, 1977, 1979). Alimentary eggs are usually laid by bees 15-25 days old. All workers in a queenright colony become fertile to such a limited extent during the nurse age (Engels & Engels, 1977).

#### Caste Specific Differences in Fertility

Comparing the vitellogenin titer and egg laying capacity in queens and queenright workers of *Scaptotrigona postica*, similarities are evident during the first two weeks of imaginal life (c. Fig. 4b and 5). After this time the queen is beginning to oviposit and continues synthesizing vitellogenin and to lay more and more eggs until daily rates of some hundreds are produced by the old physogastric queen. On the other hand, the worker after having laid one or a few alimentary eggs is becoming infertile again whilst shifting the behavioral tasks toward field

activities. Therefore, in stingless bee workers (Engels & Engels, 1977) vitellogenin is synthesized only during the nurse age like in honey bees (Engels 1972, 1974; Engels & Fahrenhorst, 1974).

*Mating Biology*

In stingless bees adult males vagabond after leaving the colony at an age of 2-3 weeks. Migrating drones are attracted by the lure of virgin queens and form aggregations or mating swarms in the vicinity of nests. In *Scaptotrigona postica* hundreds to thousands of drones are waiting on leaves (Fig. 6) or trunks close to a colony during the daytime (Engels & Engels, 1984). If a young virgin leaves for a nuptial flight, she is followed by the males.

*Queen Attractivity Bioassay*

In order to analyse at which age young queens are attractive for the males, we developed a bioassay (Engels & Engels, 1987). Fifty males taken from an aggregation are put into a mating box. After about 15 min the drones

have calmed down and are sitting on the bottom (Fig. 7a). When a gyne is added, the reaction of the males very characteristically indicates their specific interest in the female. If the virgin is highly attractive, the drones immediately start crowding and are searching for the queen (Fig. 7b). Males meeting the virgin try to copulate. In case of a less attractive queen only after some time a drone may occasionally attempt to mate. Unattractive queens are neglected by the males. In both the latter situations no crowding of the drones is observed. Queen attractivity according to these mating box bioassay data is changing drastically during imaginal life (Fig. 8). Around day 12 after emergence a virgin is most attractive to the drones. This probably is the normal mating age of a *Scaptotrigona postica* queen.

*Queen Pheromone Analysis*

Up to date in stingless bees only volatile compounds of workers have been identified which are involved in alarm release or used in scent marks placed by recruiting foragers as a

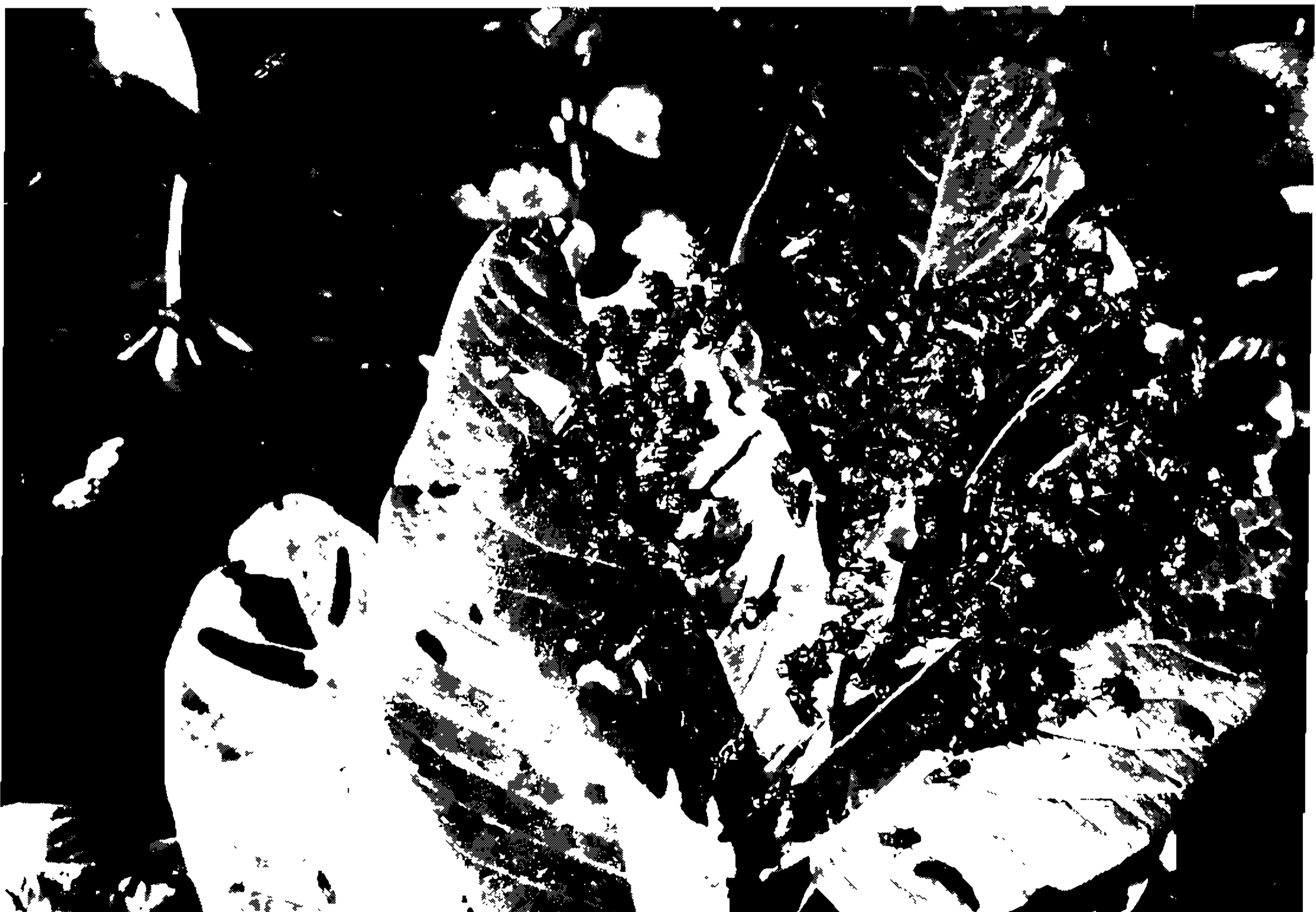


Fig. 6: Drone aggregation on a leaf in the vicinity of a colony of *Scaptotrigona postica* containing virgin queens. (Magnification 0.08 x). The males have migrated here from many nests most of them far away, thus bringing about a quasi panmictic behavior of a given breeding population. During the daytime the drones are waiting for a virgin going out for a short nuptial flight. This mating system includes reverse flying distances of males and females to the encounter site. The outbreeding strategy is pursued by the drones (Engels, 1984).

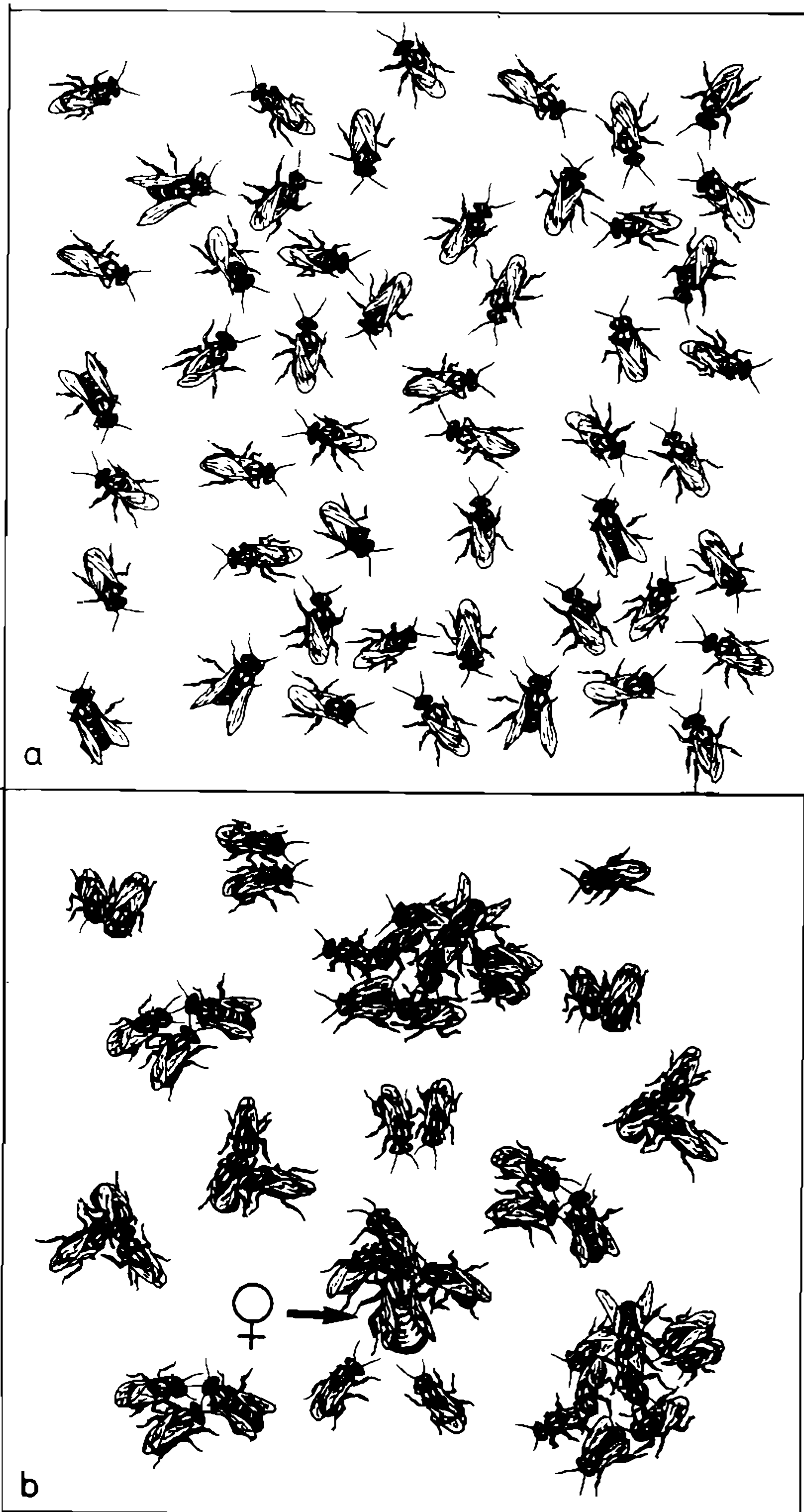


Fig. 7: Mating box bioassay to test the attractivity of a queen in *Scaptotrigona postica*. 50 drones taken from an aggregation are put into a wooden box covered by a pane of glass. a = after some minutes the males are walking or sitting on the bottom. b = immediately after introduction of a queen, the drones react by crowding and try to copulate in case of an attractive virgin (Engels & Engels, 1987).

trail pheromone (Blum, 1974; Kerr et al., 1981; Smith & Roubik, 1983). Nothing was known about queen pheromones and pheromonal functions in reproduction. The analysis of pentane extracts of *Scaptotrigona postica*, queen heads of known age and life history was surprising. There was no main compound like (E)-9-oxo-2-decenoic acid, or a chemically related compound, present as the major cephalic volatile as in queen honey bees throughout their imaginal life (c. Free, 1987). Instead of this, two patterns of compounds were found characteristic for young virgin and old egg laying queens, respectively. The bouquet of virgin queens consists mainly of secondary alcohols with 7 to 13

C atoms. In old queens these components are still present but in very low percentages. The typical compounds of the bouquet found in physogastric queens are alkenes of which tricosen is dominating (Francke, 1987).

Although biotests are still lacking, it seems as if in stingless bee queens two complexes of queen substances may exist: Perhaps the secondary alcohols are the perfume making a virgin queen attractive for the drones. Later on in the old physogastric and egg laying queen possibly the alkenes are pheromones affecting workers and colonial homeostasis (Engels et al., 1987b).

In stingless bee queens the abdominal tergite glands are well developed which probably, like in honey bees, are also a source of queen pheromones (Cruz Landim, 1987). Recent attempts to correlate the activity of these glands with ovarian functions in worker bees failed (Cunha et al., 1987).

#### Worker Pheromone Analysis

In stingless bees there is a similar division of labor as in honey bees. During an adult lifetime of 50-70 days, the first month of a worker's activities consists of indoors duties. Thereafter the house bee becomes a field bee foraging nectar and pollen. Communication capacity for scent marking and mandibular gland development was found maximal in workers 40-50 days old (Cruz Landim & Ferreira, 1968).

We can imagine that such diverse tasks require a different vocabulary in chemocom-

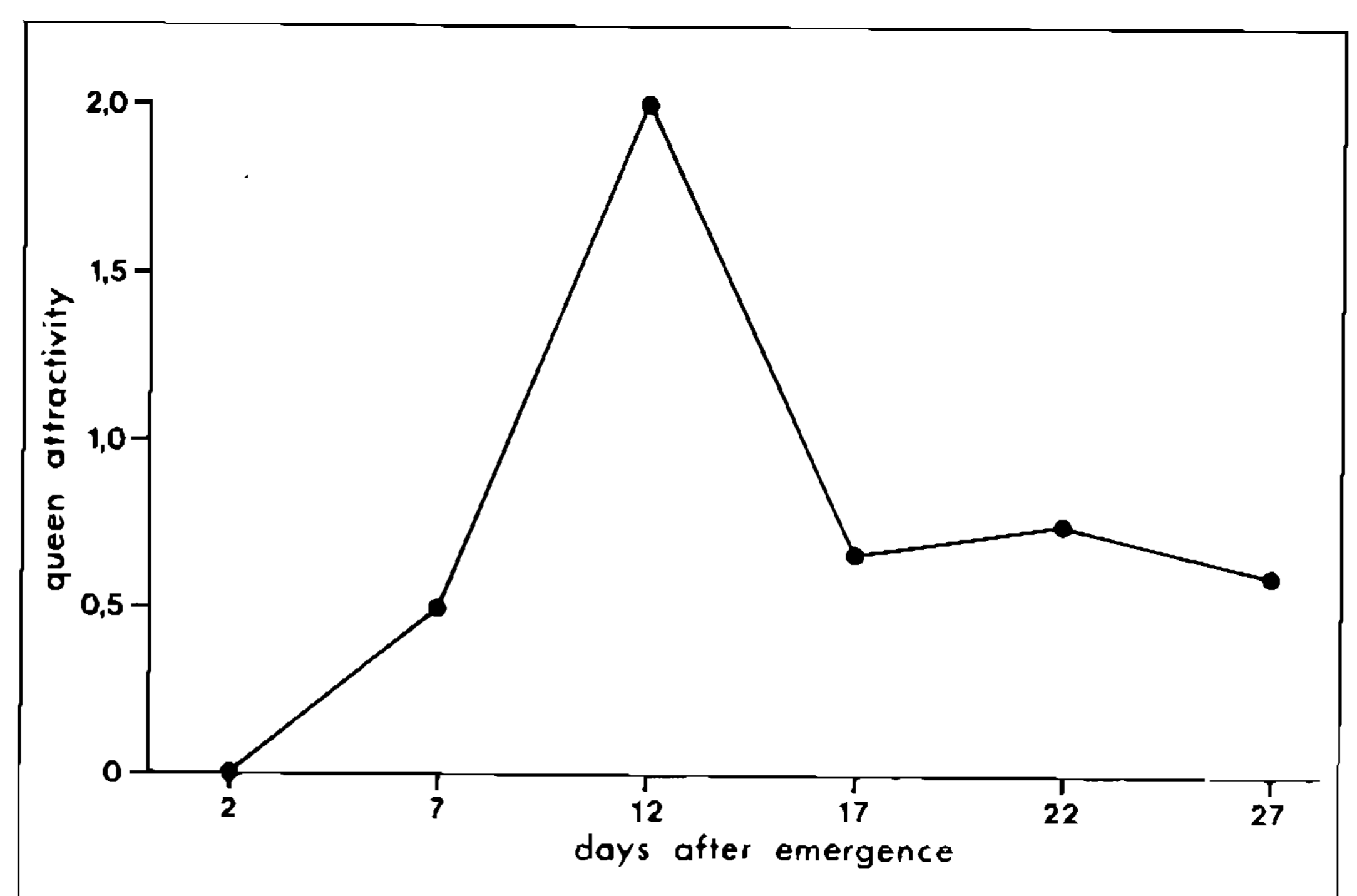


Fig. 8: Changes in attractivity of a young queen to conspecific drones during her first month of adult life in *Scaptotrigona postica*. The quotient of attractivity is calculated on the mating box biotest data scaled in 2 = maximum, 1 = medium and 0 = zero response of the males. Means of 26 bioassayed queens of different age. The highest attractiveness of a virgin is attained about 12 days after emergence and corresponds to the average mating age (Engels & Engels, 1987).

munication. Indeed the patterns of volatiles identified from *Scaptotrigona postica* worker head extracts (Francke et al., 1983) indicate the existence of specific bouquets typical for a distinct age (Table II). We can suppose that some of the compounds predominantly present in nurse bees may be of reproductive significance, because it is during the nursing phase that the workers are laying a few eggs, are provisioning brood cells, and have frequent contacts with the physogastric queen, in particular

in course of an oviposition cycle (Sakagami & Zucchi, 1963; Engels et al., 1980; Sakagami, 1982).

To date 70 cephalic volatiles are known from *Scaptotrigona postica* workers (Engels et al., 1987a). This is much more than in any other bee. In part the number of compounds depends on the analytical expense. The more sophisticated the technique the more components will be identified. But besides this, it

TABLE II

Variation in the pattern of volatiles found in adult worker bees of *Scaptotrigona postica* according to age and function as obtained by gas chromatography/mass spectrometry (Francke et al. 1983). Nurse bees are participating in reproduction by laying alimentary eggs and by provisioning the brood cells with larval food. In the nurse age the workers also have most contacts with the queen

Compounds	Newly emerged	Nurse	Garbage carrier	Guard	Pollen forager	Pattern of occurrence in adult worker ontogenesis
Dodecane	x	x	x	x	x	concentration independent on age
Tridecane	x	x	x	x	x	
Hexanol	x	x	x	x	x	
2-Nonanone	x	x	x	x	x	
Tetradecane	x	x	x	x	x	
2-Pentylhexanoate	x	x	x	x	x	
Pentadecane	x	x	x	x	x	
Undecenone	x	x	x	x	x	
2-Undecanone	xx	xx	x	xx	xx	
2-Tridecanol	xx	xx	xx	xx	xx	
2-Heptadecanone	xx	xx	xx	xx	xx	
2-Tridecanone	xxx	xxx	xxx	xxx	xxx	
2-Pentadecanone	xxx	xxx	xxx	xxx	xxx	
$\gamma$ -Decalactone	x	x				
3-Methyl-1-butanol	x	x		x	x	
2-Tridecenone(a)	xx	x	x	x	x	
2-Tridecenone(b)	xx	x	x	x	x	
Tetradecylbutanoate	xx	x	xx	xx	xx	
2-Hexanol	x		x	x	x	
3-Methylbutylhexanoate		x	x	x	x	nurse bees
2-Heptadecanol	xx	xx	x	xx	xx	
2-Undecanol	x	xx	x	xx	x	
2-Pentadecanol	x	xx	x	xx	xx	
2-Heptanone	x	x	xx	xx	xx	garbage carriers
2-Nonanol	x	x	xx	xx	xx	
Dodecylbutanoate	x	x	x	xx	x	
Benzaldehyde		x	xx	xx	xx	
Hexylhexanoate		x	xx	xx	xx	
Hexyl-3-methylbutanoate			x	x	x	guard bees
Hexyl-1-2-hexenoate			x	x	x	
2-Phenylethanol			x	x	x	
2-Heptanol	x	xx	xx	xx	xxx	pollen foragers
Z-5-Tetradecenylbutanoate	x	xx	xx	xxx	xxx	
Z-7-Hexadecenylbutanoate	x	x	xx	xxx	xxx	
2-Heptylhexanoate		x	x	x	xx	
2-Octanol, 1-Hexylbutanoate				x	x	

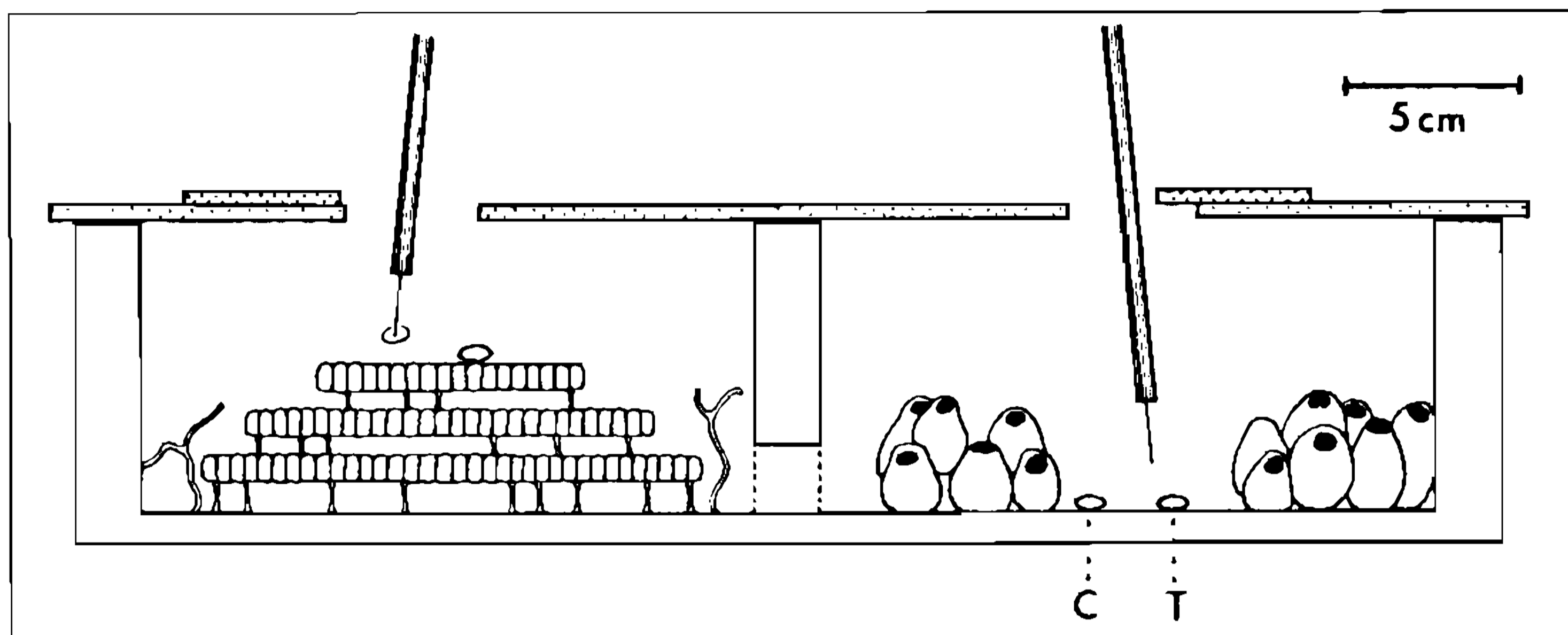


Fig. 9: Experimental nest box designed for a bioassay of *Scaptotrigona postica* worker reactions to volatiles. Small wax discs (T) impregnated with synthetic compounds according to identified components of pentane extracts of worker heads were placed aside a control disc (C). During a 3 min testing period worker reactions were recorded either in the storage area (right) or on top of the brood nest (left). Only data from the latter biotests are discussed here (c. Engels et al., 1987a).

seems that eusocial bees really make use of an extraordinary diversity of semiochemicals (Francke et al., 1984; Francke, 1987).

#### Bioassay for Worker Reactions to Volatiles

Pheromones are transmitted in the air and recognized by olfaction or in case of more involatile compounds perceived by contact perception through antennating or licking. Therefore, a bioassay designed for testing stingless bee worker reactions to distinct compounds within the nest under short range conditions has to consider both ways of possibly receiving

chemical information. As strong reactions often are reinforced by pheromones transmitted by nestmates, some confinement of the test area is desirable. Our experimental nest box is divided into two compartments in order to avoid too many disturbances (Fig. 9). Test substance solutions can be exposed on small discs punched out for bees wax either in the storage part of the nest or on top of the uppermost brood comb (Engels et al., 1987a). Here I will refer only to data collected by testing in the brood nest where young nurse bees are working.

TABLE III

Bioassay results in exposure of synthetic volatiles to worker bees on top of the brood nest in *Scaptotrigona postica* (Engels et al., 1987a). Some of the attractant compounds did also release licking, food exchange or wing beats which all are normally observed as part of worker/queen and worker/worker reactions on the combs. Bold printed figures indicate very pronounced test responses.

Worker reactions	Compound tested																								
	3-Methyl-1-butanol	1-Hexanol	2-Hexanol	2-Octanol	2-Heptanol	2-Nonanol	2-Undecanol	2-Tridecanol	Hexyl 3-methylbutanoate	Hexyl pentanoate	3-Methylbutyl hexanoate	2-Pentyl hexanoate	2-Heptyl hexanoate	3-Methylbutyl E-2-hexenoate	2-Heptyl E-2-hexenoate	Tetradecyl butanoate	Z-5-Tetradecenyl butanoate	Y-Decalacton	$\delta$ -Decalacton	2-Nonanone	Z-6-Undecen-2-one	Z-6-Tridecen-2-one	Z-8-Tridecen-2-one	Benzaldehyde	
Released on the top brood comb																									
Attraction																									
Attraction: lick disc	0	1	1	0	1	1	2	0	0.5	1	0	0	0	1	1	0	0	0.5	0	1	1	1.5	1.3	1	
Attraction: trophallaxis	0	2	0	1	0	1	1	0	0	2	1	1	0	0	0	0	0	0	0	1	0	0	0	0	
Attraction: wing beat	1	4	1	3	0	3	1	1	0.5	1	0	0	2	1	3	1	0	0	0	1	0	0	0.7	3	
Overall reaction intensity	1	7	2	4	1	5	4	1	1	4	1	1	2	2	4	1	0	0.5	0	3	1	1.5	2	4	



*Intranidal Testing of Synthetic Compounds*

The behavioral response of *Scaptotrigona postica* workers confronted with a pure synthetic volatile was very characteristic and reproducible. Generally a substance could release alarm and/or attract and/or repel the bees. Besides this, about 10 typical and specific reactions were observed, always some of them at the same time or in an immediate sequence (Engels et al., 1987a).

Here I will concentrate on those behavioral responses which were released by the 24 attractants (Table III). The workers approached the test disc and sometimes (15 of 24 compounds) began to lick it. Exchange of liquid food was initiated only by a few substances (8 of the 24 compounds), especially by alcohols and some esters. But it has to be mentioned that in stingless bees trophallaxis generally is less frequent than in honey bees (Velthuis, 1985). Wing beating during antennating the odorous disc was observed in several tests (16 of the 25 compounds). The overall intensity of these three reactions occurring in biotests with attractant compounds differed much (Table III). The amounts of pure synthetic substance offered in a single test was different with respect to absolute quantities as well as to bee head equivalents. The zero level of our reaction scale, ranging up to 5, was defined as the threshold amount which within the 3 min of a testing period did not release more reactions as the control disc near by which was blank or impregnated with pure solvent (pentane). Therefore, a high overall reaction intensity of 3 or exceeding values may indicate that the respective compound is a constituent of a reproductive pheromone. Of course a real pheromone especially in social bees will always be composed of a complex mixture of compounds, and only this bouquet will transmit the complete information.

CONCLUSIONS

Consequently volatile mixtures varying in respect to qualitative and quantitative composition have to be evaluated in future biotests in order to better understand the role of pheromones in stingless bee reproduction. As innumerable artificial bouquets can be composed using the 70 identified cephalic volatile compounds yet known in *Scaptotrigona postica* workers, we need some instructions for setting up meaningful mixtures. The most promising way to get such informations seems to be the

analysis of the natural pheromones themselves. New sampling techniques hopefully will enable us to realize a more direct decoding of the chemical language used in the context of reproduction and other functions by social bees like the stingless bees.

Although our knowledge on the real composition of reproductive pheromones in stingless bees is still very incomplete, and today nothing is known for instance about primer functions of a queen pheromone, some contours of a picture illustrating the highly complex social structure of the biology of reproduction in stingless bees already became visible during the past investigations. Comparing known queen functions in honey bee and stingless bee colonies, quite a number of striking differences have to be noticed especially concerning queen/worker relations (Table IV).

An adult honey bee queen is more or less permanently surrounded by a retinue mainly formed by nurse-aged workers feeding, palpat-ing and licking the queen. Court formation is not consistently observed in stingless bees. Only occasionally at the beginning of an ovipositioning cycle is the queen fixed by the workers forming a court (Sakagami & Zucchi, 1963; Engels et al., 1980). The workers normally do not lick the queen.

In honey bees (cf. Free, 1987) many inhibiting effects on the workers are known to be caused by the queen substance (c. Table I) which mainly concern rearing new queens and worker fertility (Engels, 1986, 1987). In stingless bees both occur as a normal event in queenright colonies. Dominating the colony in *Apis* is effected by the same queen pheromone which also serves as a female sex lure in mating. In stingless bees, the data available today (c. Table

TABLE IV

Summary of queen/worker interactions in stingless bee reproduction and age dependent changes in queen cephalic volatiles as found in *Scaptotrigona postica* (Engels et al., 1987b; Francke, 1987)

No pronounced court formation and licking by workers	
No inhibition of:	construction of queen cells worker ovary functions worker egg laying
Stimulation of:	provisioning by workers of brood cells production of alimentary eggs
Main volatile head compounds:	no decenoic acids varying bouquets according to age secondary alcohols in young queens alkenes in old physogastric queens
Mating biology:	drone attraction by young queens

IV) indicate that probably the sex pheromone transmitted by young virgins, to attract drones, and the queen substance, released within the brood nest by old egg laying and physogastric queens, are chemically different bouquets without a common main compound. Such a concept of two complexes of queen substances, functional in stingless bee queens according to age and mating, has to be verified in more detail by future research. If this hypothesis will be substantiated, then in stingless bees socioevolution, queen pheromones used for stabilizing colonial homeostasis must have been newly evolved in attaining an eusocial level. In honey bees evidently the original female sex pheromone became multifunctional in course of a colonial organization. This interpretation would support the idea of a fairly independent eusocial evolution in the bee subfamilies *Meliponini* and *Apini* (cf. Winston & Michener, 1977) from quite another point of view. Queen pheromones, like a number of other problems of reproduction, appear to be even more complex and complicated in stingless bees than in honey bees, thus offering many aspects for further comparative studies.

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#### REFERENCES

- AKAHIRA, Y.; SAKAGAMI, S.F. & ZUCCHI, R., 1970. Die Nähreier von den Arbeiterinnen einer Stachellosen Biene *Trigona (Scaptotrigona) postica*, die von der Königin kurz vor der eigenen Eiablage gefressen werden. *Zool. Anz.*, 185 :85-93.
- BLUM, M.S., 1974. Pheromonal Bases of Social Manifestations in Insects. p. 190-199. In: BIRCH, M.C. (ed.): Pheromones. Elsevier, New York.
- CUNHA, M.S.; CUNHA, R.A. & PIMENTEL, M.L., 1987. Interactions between Corpora allata Development of Workers and Pheromones-Producing Glands Activity of Queens of *Frieseomelitta silvestrilanguida* (Hymenoptera, Apidae). Abstr. Int. Symp. Insect Physiology, Biochemistry and Control, Rio de Janeiro, p. 13.
- CRUZ-LANDIM, C. de, 1987. Morphology of Pheromone Glands in *Meliponinae* (Hymenoptera, Apidae) p. 441-442. In: EDER, J. and REMBOLD, H. (eds.): Chemistry and Biology of Social Insects. Verlag J. Peperney, München.
- CRUZ-LANDIM, C. & FERREIRA, A., 1968. Mandibular Gland Development and Communication in Field Bees of *Trigona (Scaptotrigona) postica* (Hymenoptera: Apidae). *J. Kans. Entomol. Soc.*, 41 :474-481.
- ENGELS, E. & ENGELS, W., 1984. Drohnen-Ansammlungen bei Nestern der Stachellosen Biene *Scaptotrigona postica*. *Apidologie*, 15 :315-328.
- ENGELS, E. & ENGELS, W., 1987. Age Dependent Queen Attractiveness for Drones and Mating in the Stingless Bee, *Scaptotrigona postica*. *J. Apicult. Res.*, in press.
- ENGELS, E.; ENGELS, W.; SCHRÖDER, W. & FRANCKE, W., 1987a. Intradial Worker Reactions to Volatile Compounds Identified from Cephalic Secretions in the Stingless Bee *Scaptotrigona postica* (Hymenoptera, Meliponinae). *J. Chem. Ecol.*, 13 :371-386.
- ENGELS, E.; ENGELS, W. & FRANCKE, W., 1987b. Do Stingless bees have two Complexes of Queen Substance? p. 710-711. In: EDER, J. and REMBOLD, H. (eds.): Chemistry and Biology of Social Insects. Verlag J. Peperney, München.
- ENGELS, W., 1972. Quantitative Untersuchungen zum Dotterprotein-Haushalt der Honigbiene *Apis mellifica*. *Wilh. Roux, Arch.*, 171 :55-86.
- ENGELS, W., 1973. Das zeitliche und räumliche Muster der Dottereinlagerung in die Oocyte von *Apis mellifica*. *Z. Zellforsch.*, 142 :409-430.
- ENGELS, W., 1974. Occurrence and Significance of Vitellogenins in Female Castes of Social Hymenoptera. *Amer. Zool.*, 14 :1229-1237.
- ENGELS, W., 1986. The Concept of Chemical Communication in Arthropods as Realized in Social Bee Reproduction. *Adv. Invert. Reprod.*, 4 :285-296.
- ENGELS, W., 1987. Reproduction and Caste Development in Social Bees. p. 275-281. In: EDER, J. and REMBOLD, H. (eds.): Chemistry and Biology of Social Insects. Verlag J. Peperney, München.
- ENGELS, W. & ENGELS, E., 1977. Vitellogenin und Fertilität bei Stachellosen Bienen. *Ins. Soc.* 24 :71-94.
- ENGELS, W.; ENGELS, E. & LOTZ, G., 1980. Nestbiologie der Stachellosen Biene *Scaptotrigona postica*. IWF Film No. C 1351. Göttingen.
- ENGELS, W. & FAHRENHORST, H., 1974. Alters- und Kastenspezifische Veränderungen der Hämolymp-Phosphor-Spektren bei *Apis mellifica*. *Wilh. Roux' Arch.*, 174 :285-296.
- FRANCKE, W., 1987. Allgemeine und Spezielle Muster in der Chemie komplexer Hymenopteren-duftstoffe. Abstr. IUSSI - Meeting, Bayreuth, p. 13.
- FRANCKE, W.; ENGELS, E.; ENGELS, W. & ROSENGREN, R., 1987. Chemical News from Bees and Ants. p. 430-431. In: EDER, J. and REMBOLD, H. (eds.): Chemistry and Biology of Social Insects. Verlag J. Peperney, München.
- FRANCKE, W.; SCHRÖDER, W.; BERGSTRÖM, G. & TENGÖ, J., 1984. Esters in Volatile Secretion of Bees. *Novae Acta Regiae Societatis Scientiarum Upsaliensis, Serie V:C*, 3 :127-136.
- FRANCKE, W.; SCHRÖDER, W.; ENGELS, E. & ENGELS, W., 1983. Variation in Cephalic Volatile Substances in Relation to Worker Age and Behavior in the Stingless Bee *Scaptotrigona postica*. *Z. Naturforsch.*, 38c :1066-1068.
- FREY, J.B., 1987. Pheromones of Social Bees. Chapman and Hall Ltd., London.

- KERR, W.A.; BLUM, M. & FALES, H.M., 1981. Communication of Food Source between Workers of *Trigona spinipes*. *Rev. Bras. Biol.*, 41 :619-623.
- NOGUEIRA-NETO, P., 1970. A Criação de Abelhas Indígenas sem Ferrão. Edição Tecnapis, Editora Chácaras e Quintais, São Paulo, Brasil.
- RUTTNER, F., 1985. Reproductive Behavior in Honey Bees. p. 225-236. In: Fortschritte der Zoologie 31, HÖLLDOBLER, B. and LINDAUER, M. (Hrsg.): Experimental Behavioural Ecology. G. Fischer Verlag, Stuttgart, New York.
- RUTTNER, F., 1987. Biogeography and Taxonomy of Honey Bees. Springer Verlag. Berlin, Heidelberg, New York.
- SAKAGAMI, S.F., 1966. Techniques for the Observations of Behaviour and Social Organization of Stingless Bees by using a Special Hive. *Pap. Avuls. Dep. Zool. São Paulo*, 19 :151-162.
- SAKAGAMI, S.F., 1982. Stingless Bees. p. 362-423. In: HERMANN, H.R. (ed.): Social Insects, Vol 3, Academic Press, New York.
- SAKAGAMI, S.F.; BEIG, D.; ZUCCHI, R. & AKAHIRA, Y., 1963. Occurrence of Ovary-Developed Workers in Queenright Colonies of Stingless Bees. *Rev. Bras. Biol.*, 23 :115-129.
- SAKAGAMI, S.F. & ZUCCHI, R., 1963. Oviposition Process in a Stingless Bee, *Trigona (Scaptotrigona) postica* (Hymenoptera). *Studia Entomol.*, 6 :497-510.
- SMITH, B.H. & ROUBIK, D.W., 1983. Mandibular Glands of Stingless Bees (Hymenoptera, Apidae): Chemical Analysis of their Contents and Biological Function in two Species of *Melipona*. *J. Chem. Ecol.*, 9 :1465-1472.
- STAURENGO da CUNHA, M.A., 1977. Ovarian Development in *Scaptotrigona postica* Latr. (Hymenoptera: Apidae) I. Morphological Study. *Dusenía*, 10 :205-215.
- STAURENGO da CUNHA, M.A., 1979. Ovarian Development in *Scaptotrigona postica* Latr. (Hymenoptera: Apidae) II. A Quantitative Study. *Ins. Soc.*, 26 :196-203.
- VELTHUIS, H.H.W., 1985. The Honeybee Queen and the Social Organization of her Colony. p. 343-357. In: Fortschritte der Zoologie 31, HÖLLDOBLER, B. and LINDAUER, M. (Hrsg.): Experimental Behavioural Ecology. G. Fischer Verlag, Stuttgart, New York.
- WINSTON, M.L. & MICHENER, C.D., 1977. Dual Origin of Highly social Behaviour among Bees. *Proc. Natl. Acad. Sci. U.S.A.*, 74 :1135-1137.