Reproductive behaviour of stingless bees: nest departures of non-accepted gynes and nuptial flights in *Melipona favosa* (Hymenoptera: Apidae, Meliponini)

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We report here for the first time that the numerous ‘superfluous’ virgin queens in colonies of *Melipona* stingless bees are not all killed by their sister workers. ‘Excessive’ production of gynes is a typical feature of reproductive behaviour in *Melipona*. Until now the view prevailed that surplus *Melipona* gynes, not needed for supersedure or for swarming, are all killed by the workers. Aggressive worker behaviour towards gynes is very common in *Melipona*. Very few gynes have a chance to participate in queen replacement or in colony division through swarming, since colony mortality is low and swarming and queen supersedure are rare events. Therefore, the fact that numerous virgin queens are produced daily requires further investigation. We studied the acceptance of a virgin queen, followed by the nuptial flight, in colonies which had been experimentally dequeened. The nuptial flight was just a few minutes, taking from two minutes and 30 seconds to eight minutes. Subsequently, we studied the behaviour of gynes in colonies with laying queens. We did this by closely observing the behaviour of nest-departing bees and by using a device that intercepted non-accepted gynes as they departed from the nest. We established that 57% of the experimentally marked gynes left the nest alive. This observation contradicts the currently held view concerning the destiny of virgin queens in *Melipona*. We discuss the implications of the finding that non-accepted gynes leave the nest in order to escape from worker aggression. It is hypothesised that ‘chasing’ sister gynes out of the nest is a strategy whereby workers improve their own inclusive fitness.

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Introduction

The position of stingless bees

Unlike most bee species that live solitary lives, the stingless bees (Apidae, Meliponini) and the true honeybees (Apidae, Apini) are highly social (‘eusocial’) and have complex and long-lasting colonies (Michener 1974). The Meliponini group is more diverse (hundreds of species) than the Apini group (five to ten species). The smallest species of stingless bees measure only about 3 mm and their very small nest fits into a cigarette packet. The largest stingless bees are as big as the honeybee (*Apis mellifera*) and the total volume of the nest of certain stingless bee species may be more than 200 litres. From C.D. Michener’s recent comprehensive compilation of the taxonomy of the world’s bees (Michener 2000) it appears that the taxonomy of several groups of the Meliponini has hardly been studied at all. Stingless bees are pantropical and in all their regions their principal function is to pollinate the tropical forest. This status is particularly dominant in the American tropics and Australia. Prior to the commercial introduction and subsequent spread of the honeybee, stingless bees were the only eusocial bees in these continents. Due to the complex social organisation and the diversity of social behaviour within this taxon, stingless bees are an excellent model group on which to base evolutionary studies of social behaviour. Because they are natural pollinators and vary so much in size and foraging behaviour, these bees have recently received much attention as possible pollinators of agricultural crops, particularly in crops grown in greenhouses.

Stingless bees have been used by man for many centuries. In the American tropics a well-developed system of beekeeping for the production of honey and wax was practised by the ancient Maya cultures of Mesoamerica. Today, in Yucatán, Mexico, remnants of this once very important tradition still prevail. All over Central America stingless bees, mainly the large-sized *Melipona* species, are still kept by rural people (figure 1). In the Amazonian region the traditional use of stingless bees is characterised by sophisticated systems of 'honey hunting' which can include the 'management' of colonies in their natural nesting sites. Over the last few decades applied research and fundamental biological studies have led to various activities and projects being focused on this very interesting group of bees.

Figure 1. Traditional stingless beekeeping in Central America (El Salvador). The hives consist of hollow tree-trunks in which the nests had been found in the forest. The hives are generally suspended under the eaves of the houses. Both ends of the hives are closed with stoppers that can removed for the harvest. Photo: M.J. Sommeijer.
Traditionele bijenteelt in Centraal-Amerika (El Salvador). De bijenwoningen bestaan uit de stukken boomstam waarin de nesten in het bos zijn gevonden. Deze worden onder de overhangende daken van de woningen gehangen. De open einden van holle stam worden afgesloten met grote stoppen die tijdens de oogst worden verwijderd.

Typical characteristics of stingless bees

The group of stingless bees differs in several respects from the honeybee. The morphological characteristics of stingless bees include wing venation, rudimentary structure of the sting, strong mandibular musculature and the dorsal position of the wax glands (ventral in Apini).

Most species build their nests in existing cavities like hollow trees or in the ground. A few species build their nests in exposed positions. The narrow and often elaborate entrance to the *Melipona* nest lets the nest to be defended by one or more guards positioned in the outside opening of the tube-shaped entrance. Within the nest there is a clear separation between the brood chamber and the food storage area. Storage pots are several times larger than brood cells (figure 2). In general pots with different contents are not kept separate: pots containing honey intermingle with those containing pollen. In most species brood cells are arranged in horizontal combs but they may also be built in clusters. In all species with horizontal combs (all *Melipona* spp. and most *Trigona* spp.), the compact stack of combs is surrounded by waxy sheets (involucrum). Figure 3 gives a general view of a complete nest of the stingless bee *Tetragonisca angustula*. Species arranging brood cells in clusters do not have an involucrum. This means that these species can use irregularly shaped cavities for nest building (Michener 1974).

Reproductive behaviour in the nest is typical. Whereas the Apini are progressive provisioners of their larvae, the Meliponini have a system of mass-provisioning brood cells. This means that all food needed for the complete larval development is brought into the cell prior to oviposition. New bees are reared from eggs that are oviposited in brood cells immediately after the cell has been provided with larval food. During very short periods of high activity, a small number of bees deposit liquid food in the brood cell; immediately after this, an egg is laid on top of the food. In all species these characteristic bouts of integrated provisioning and oviposition, known as the 'Provisioning and Oviposition Process (POP), alternate with long periods of cell-building behaviour (figure 4). Details of POP behaviour, including the division of labour among the workers, have been studied by Sakagami and co-workers (review in Sakagami 1982) and Sommeijer and co-workers (Sommeijer 1984, Sommeijer & de Bruijn 1984, Sommeijer *et al.* 1984, Sommeijer *et al.* 1985).

Colony reproduction takes place through swarming. In stingless bees this process is still poorly understood. A few observations of species other than *Melipona* indicate that there is long-lasting contact between the mother nest and the filial nest (Sakagami 1982, Van Veen 1999, Van Veen & Sommeijer 2000). This implies that daughter colonies can only be established within the flight range of the mother nest. During our intensive observations of numerous colonies of various *Melipona* species in their natural habitat, we have never seen obvious symptoms of swarming. Although swarming in *Melipona* may occur inconspicuously, we consider it to be infrequent.

Laying workers and the role of males and queens in reproduction

In contrast to the honeybee, where the queen exerts pheromonal and behavioural control over worker reproduction, most stingless bees have laying workers which commonly feed the queen by laying trophic worker eggs. Recently a number of articles have concentrated on the contribution that workers make to the production of males by the laying

Figure 2. Storage pots in a nest of *Melipona*. Details of storage pots for honey and pollen. Photo: M.J. Sommeijer.
Voorraadpotten in het nest van Melipona. *Detail van de opslagpotten voor honing en stuifmeel.*

of reproductive worker eggs (Sommeijer *et al.* 2003b, Sommeijer *et al.* 1999, Sommeijer & van Buren 1992, Chinh *et al.* 2003 in press; Koedam *et al.* 1999, Van Veen *et al.* 1997, Kellie *et al.* 2002, Paxton *et al.* 2001; Peters *et al.* 1999, Ratnieks & Reve 1992, Toth *et al.* 2002).

The males ('drones') have no specific tasks inside the nest. Outside, they inseminate virgin queens (gynes) that replace the mother queen ('supersedure') or establish a daughter colony. In thriving colonies of *M. favosa* drone production is based largely on the laying of reproductive eggs by workers (Sommeijer *et al.* 1999, 2003b, Chinh *et al.* 2003). In other species a considerable number of the males are sons of the queen (Paxton *et al.* 2001). Males are produced regularly all through the year. In *M. favosa* it has been established that at colony level they are produced in distinct bouts (Sommeijer *et al.* 2003b, Chinh *et al.* 2003). These periods of drone production can last as long as several weeks and alternate with periods in which there is no drone production. In our observations of the behaviour of *M. favosa* males, we found that they leave the nest at an average age of 18.6 days (Van Veen *et al.* 1997) and do not return. In the daytime, males congregate at distinct drone congregation sites (DCS's) and they remain at these sites for several days (Sommeijer & De Bruijn 1995, Van Veen *et al.* 1997). Because males are independent of the nest, they may disperse and spread their genes over a great distance. Reproduction chances for individual drones are restricted by the fact that queens of *Melipona* mate with only one male (*Apis* queens mate with 10-20 males).

After queens have started egg laying they do not leave the nest again. Daughter colonies are always established with a 'gyne' (virgin queen). The mother queen is not often replaced by one of her daughters because laying queens in *Melipona* live for a long time. From our observations it appears that laying *Melipona favosa* queens may stay active in the same colony for 3 or more years. So far there are no firm data on the flight range of the gyne during the nuptial flight, but we assume that the range is limited.

'Excessive' gyne production in Melipona and the elimination of superfluous gynes

The species of the genus *Melipona* differ from the other stingless bee genera particularly by their system of queen production: *Melipona* queens are reared in the standard type of brood cell and are produced continuously and in large numbers. In *M. favosa* 5.1% of all emerging females are gynes, and in *M. trinitatis* the proportion is 4.8% (Sommeijer *et al.* 2003b). There is no significant seasonal variation in gyne production and this results in the daily emergence of gynes. Hitherto, all publications about queen production and gyne behaviour in *Melipona* have maintained that all the non-accepted gynes are killed by the workers within days. In their review on gyne behaviour Imperatriz-Fonseca and Zucchi (1995) state that "the emerging virgin queens ... may be killed, supersede the dominant queens, or swarm to start a new nest with workers". The rare occurrence of these two reproductive opportunities considered in *Melipona* however allows for reproduction by only a very small proportion of the gynes. A recent discussion about queen production in *Melipona* by Ratnieks (2001) is also still based on the general view that young queens can only contribute to reproduction in two ways: by participating in the replacement of the mother queen and by swarming.

Figure 3. General view of a nest of stingless bees. The small species *Tetragonisca angustula* is abundant and commonly kept in Costa Rica. The nest is built in cavities of various materials, even in cardboard boxes. The illustration shows an opened hive with horizontal brood combs, the waxy envelopes (involucrum) around the brood unit and the clustered food pots containing pollen and honey. The honey is claimed to be medicinal. Photo: M.J. Sommeijer.

Overzicht van een nest van angelloze bijen. De kleine zeer algemene soort Tetragonisca angustula *bouwt nesten in allerlei holtes. Deze soort wordt vaak in kastjes gehouden in Costa Rica. In dit geopende bijenkastje zien we dat het broednest bestaat uit horizontale raten, omgeven door het 'involucrum' van waslamellen. Daarbuiten zijn de voorraadpotten met honing en stuifmeel in clusters gerangschikt. De honing heeft in dit gebied grote medicinale betekenis.*

Our objective: to study the fate of non-accepted gynes

Motivated by the widely held view concerning the 'wasteful' over-production of gynes in *Melipona* and by our observation of numerous gynes at drone congregations (Sommeijer & De Bruijn 1995), we hypothesise that *Melipona* gynes may contribute to reproduction in yet another way. Fundamental to our hypothesis is our new assumption that not all non-accepted gynes are killed and that a number of them may leave the nest alive. Therefore we studied gyne behaviour in great detail. In this paper we present the results of observations and experiments on the flight behaviour of gynes from queenright nests (with laying queens) and from nests from which the queen had been experimentally removed. We focus principally on the prenuptial behaviour and particularly on the departure of gynes from the nest. The extranidal behaviour of non-accepted gynes will be dealt with in another paper.

Material and methods

Observations were carried out in Trinidad and Tobago, West Indies, during various series of observations made between 1994 and 2000. All *M. favosa* colonies used were housed in observation hives and installed in dark rooms. Colonies had undisturbed flight to the outside via transparent plastic tubes leading through the wall. Using red light conditions we could observe undisturbed intranidal behaviour. Colony size varied from a few hundred bees to a maximum of 500, which is normal for this species.

Queen acceptance was studied in 19 queenless colonies, obtained either by dividing colonies or by removal of the laying queen. Soon after they emerged from the cell, gynes were marked with a coloured waterproof felt-tip pen. To collect and mark gynes with minimal disturbance, we applied the standard procedure that we developed over the years at Utrecht University for age-marking *Melipona* bees. For this,

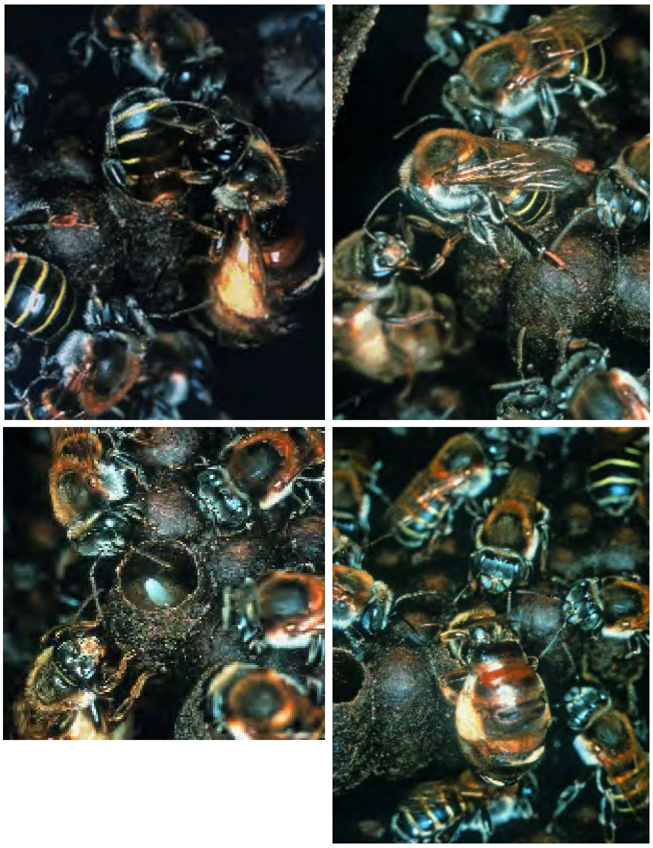

Figure 4.Provisioning and Oviposition Process, or POP, in *Melipona*. The pictures show subsequent steps of the typical behavioural sequence: **A**. A worker inserts her complete body into the brood cell in order to discharge larval food from the crop, while the queen is drumming and antennating the worker to stimulate her to release the food. **B**. A worker in laying position to place an egg on the surface of the larval food. **C**. The worker egg is visible on the surface. **D**. The queen feeds on the trophic worker egg before she herself lays an egg in the same cell. Photo: M.J. Sommeijer.
Het typische Provisioning and Oviposition Process, POP, bij Melipona. *De foto' s tonen opeenvolgende stappen uit de typische gedragsketen:* ***A****. Een werkster duikt diep in de cel voor het uitpersen van de inhoud van de honingmaag terwijl de koningin deze werkster betrommelt als stimulus voor het uitspugen van het vloeibaar voedsel.* ***B****. Een leggende werkster zet een ei af op de vloeistofspiegel.* ***C****. Het werksterei staat op het voedsel.* ***D****. De koningin eet het werksterei op vlak voor haar eigen eileg in dezelfde cel.*

the mature brood combs are partially separated from the major brood-nest by placing them in small annex emergency boxes with glass covers. This annex is connected to the major observation nest box by means of a short plastic tube. Newly emerged bees generally remain on their comb for about one day after emergence and can be collected without disturbance of the behaviour in the brood nest. However, adult workers that are necessary for activities on the emerging comb can readily pass through the plastic tube between the annex and the major compartment. We recorded and marked the emerged adults twice daily and introduced them into the main brood-nest.

After removal of the laying queen, a colony was inspected at least twice a day. On each occasion we monitored the presence of gynes and their behaviour and that of interacting workers. So that we could witness the nuptial flight, the flight tube of colonies in which an accepted gyne was present was observed from 8:00 until 15:30 h. for three to seven days following acceptance, After the day of the nuptial flight, the colony was inspected every day to determine the state of the queen. At 45 days after the first queen oviposition, the emerging offspring was sexed in order to check whether the young queen had been inseminated (non-inseminated queens would produce only haploid males).

During a period of five days, a total of 23 gynes from eight queenright colonies were wing-clipped and six of these gynes were marked individually immediately after emergence. (Earlier, we had established that wing-clipping of gynes had no long-lasting influence on their behaviour or on the manner in which workers interacted with them.) This procedure enabled us to check whether they did in fact leave the nest. To record the actual departure of gynes we used a trapping device for wing-clipped gynes consisting of a funnel with a small collecting bottle placed below the exit from the colonies (figure 5). The bottles were inspected throughout the day so that we could check if the collected gynes had left the hive alive or if they had been thrown out after being killed by the workers.

Results

Presence and longevity of gynes

In 19 colonies that we used to record how many live gynes were present at the same time in one colony, we found that the number ranged from 0-15. Larger colonies usually contained more virgin queens than smaller colonies. The maximum lifespan of a non-accepted virgin queen in the colony was 14 days. In one strong colony where 32 gynes were monitored individually, the average life span of the gynes in the colonies was 5 days.

Aggression by workers towards gynes and queen acceptance

Worker aggression towards gynes was found to be very common but varied in severity. Non-accepted gynes are not always attacked the moment they emerge nor are they attacked constantly. Gynes may be left undisturbed for some time (even days) until aggressive behaviour towards them starts or continues. When aggression starts, it may be directed towards a few gynes that are present at the same time.

Queen acceptance in queenless colonies where gynes are born

A gyne was accepted 1 to 6 days after dequeening. The exact age of accepted virgin queens is known only in two cases, each queen being one day old when accepted. On the day of acceptance and on the following day all other gynes were killed. After this, some virgins were found alive in the colony in addition to the newly accepted queen.

Non-accepted gynes do depart from the nest

We noticed that non-accepted gynes were able to leave the nest. Three non-accepted gynes from different colonies were seen to pass quickly through the nest exit and fly out. These initial observations were made on days when we recorded the nuptial flight in colonies with a recently accepted queen. Since all non-accepted virgins present at the time of the nuptial flight are generally killed, we first considered this to be a now-or-never attempt to escape from worker aggression when the colony was in this particular state. However, in our subsequent experiments with wing-clipped gynes in colonies

Table 1. Recorded departures of non-accepted gynes from the mother nest. Thirteen of the 25 wing-clipped gynes were collected in the funnels. They were all in good condition. The time of departure was recorded by permanent observation of the funnels. The age of four gynes at the moment of departure was known. The other gynes were only wing-clipped in order to minimise the risk of disturbance.
Het uitvliegen van niet-geaccepteerde jonge koninginnen uit de moederkolonie. Van 25 vleugelgeknipte jonge koninginnen werden er dertien opgevangen in de trechters. Deze waren allemaal in goede conditie. Het tijdstip van vertrek uit de kolonie werd door constante observatie van de trechters vastgesteld. Van vier vertrekkende jonge koninginnen kon de leeftijd worden vastgesteld. De meestejonge koninginnen waren, om hun gedrag zo weinig mogelijk te verstoren, niet op leeftijd gemerkt.

date	colony	time (h:min)	age (days)
98/07/30	T7	12:00	unknown
98/07/30	B1	15:15	unknown
98/07/31	T2	08:50	unknown
98/08/01	B1	13:10	unknown
98/08/02	B2	10:05	2
98/08/02	B2	10:30	2
98/08/02	B2	10:45	2
98/08/02	T2	14:30	1
98/08/02	T7	14:30	unknown
98/08/02	B3	14:45	unknown
98/08/03	B3	09:55	unknown
98/08/03	B3	10:20	unknown
98/08/03	B3	16:05	unknown

with laying queens, we established that gynes regularly depart from thriving queenright colonies. Of the 23 gynes that were wing-clipped during a period of five days we saw thirteen (57%) leaving the nest alive. These thirteen originated from five of the eight colonies in which gynes had been wing-clipped. Of the six age-marked gynes, four were seen to depart. Three of these gynes were two days old and one was only one day old at the moment of departure. Non-accepted gynes thus depart from the nest throughout the day (table 1).

A comparison of the nest-departing behaviour of accepted and non-accepted gynes

Accepted gynes that departed for their nuptial flight displayed very ambivalent behaviour at departure. Hours before their departure, these gynes actively interacted with workers; they also ran through the nest, gradually moving further away from the brood nest, until finally they reached the exit-tube of the hive. They visited the exit-tube three to seven times and spent long time in the exit-tubes before finally departing for the nuptial flight. Such pre-flight stays in the exit-tube lasted up to 30 minutes. When preparing to leave the hive for the nuptial flight, gynes walked to the outside exit of the tube and clung to the mouth of the tube for a few seconds, facing the opening. They then flew off backwards, making increasing zigzag movements while keeping their head directed towards the entrance. Such typical pre-flight behaviour did not occur in non-accepted gynes. Their departure from the nest was very different. Non-accepted gynes passed through the exit-tube very quickly, took off abruptly without any sign of orientation and never returned.

Nuptial flights

We observed 21 nuptial flights; of these the flight duration was recorded 14 times. On four occasions the nuptial flight was recorded simultaneously by a second observer outside the laboratory. The results of the observation of nuptial flights are listed in table 2. Virgin queens undertook their nuptial flight between 10:01 and 13:08 h. three to seven days after their acceptance. They returned after one and a half to eight minutes. Upon inspection of the brood after 45 days we found that all colonies contained female offspring, which confirmed that all gynes had been inseminated. None of the returned gynes left the hive for a second time and they were never seen in the entrance tube again. From the duration of the flight we estimated that nuptial flight ranges could not have been much longer than 350-1500 metres.

Table 2. Nuptial flights: date, time of day, duration of flight, air temperature and age of the gyne. Standard deviation is given between brackets.
Bruidsvluchten: datum, tijdstip, duur van de bruidsvlucht, temperatuur en leeftijd van de jonge koningin. Standaarddeviatie staat tussen haakjes.

date	time (h:min)	duration (min)	temp. (ºC)	age (days)
93/10/03	11:39	5’16	32	5
93/10/08	10:29	4’14	30	unknown
93/11/17	10:01	no return	28	4
93/11/23	10:33	3’20	28	unknown
93/12/14	10:54	4’53	28	unknown
94/01/07	12:42	4’31	30	unknown
94/01/13	11:42	1’30	30	unknown
94/01/17	13:04	3’50	35	unknown
94/01/20	11:24	5’04	29	unknown
94/01/30	13:08	5’45	32	unknown
94/02/23	10:29	6’43	29	unknown
94/02/23	11:36	1’31	32	unknown
95/11/27	11:45	4’00	unknown	unknown
98/01/05	12:50	8’12	unknown	unknown
interval	10:01-13:08	1’30-8’12	28-35	
average	11:29 (1:16)	4’31 (1’51)	30.3 (2.1)	

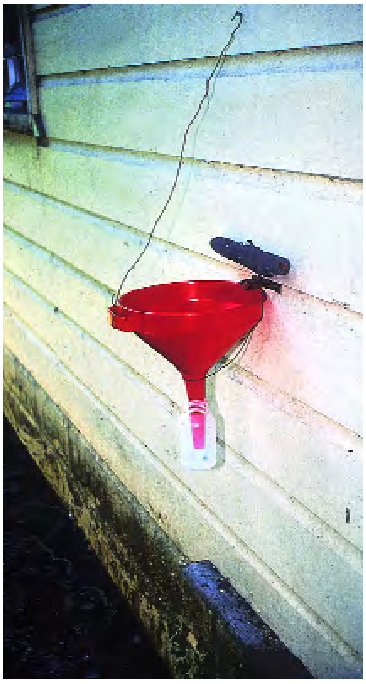

Figure 5. Method for catching the nest-departing non-accepted gynes: Funnel with bottle for collecting departing wing-clipped gynes placed under the exit of an observation hive. Photo: M.J. Sommeijer.
Methode voor het onderscheppen van de nestverlatende niet-geaccepteerde maagdelijke koninginnen. Met een trechter en een opvangflesje worden de vleugelgeknipte jonge koninginnen die uit het nest willen vliegen opgevangen.

Discussion

Gene dispersal by the nuptial flight of superseding daughters

Nuptial flights are a rare event in *Melipona* since queens are long-lived and remain in the same colonies for several years and also because swarming occurs very infrequently. We were able to observe the process of gyne acceptance and subsequent nuptial flight behaviour by dequeening thriving colonies. In all of these colonies a gyne was accepted and afterwards inseminated during a nuptial flight. The short duration of the nuptial flights indicates that mating queens do not fly very far. After mating, superseding gynes have to return to the mother nest. When gynes are making nuptial flights from a newly established colony this new colony will still be within flight range of the old colony.

Non-accepted gynes leave the nest frequently

We report here for the first time that gynes that are not accepted by their own colony leave the nest. Although these departures are common, it is not surprising that this behaviour has not been seen before. The secretive manner in which non-accepted gynes depart makes this behaviour very difficult to observe. Through detailed intranidal observations and the use of a device to collect departing wing-clipped gynes, we were able to establish that half of the non-accepted gynes leave the nest alive.

Gene dispersal through nest departure of non-accepted gynes

Our observation of nest-departing non-accepted gynes may increase our understanding of some other behavioural characteristics of *Melipona*. For example, worker aggression towards gynes can now be interpreted as having a function related to reproduction. Worker aggression causes virgin queens to leave the nest. It is important to note that worker attacks on gynes are not immediately lethal (Koedam *et al.* 1995, Silva *et al.* 1972, Van Veen *et al.* 1999). Worker aggression can take days and involves the refusal by workers to feed gynes trophallactically. This provides gynes with many opportunities to escape from the nest.

Worker aggression towards sister gynes enhances worker inclusive fitness

Until now little attention has been given to the fact that worker aggression directed towards gynes is generally of long duration and is often interrupted. The numerous gynes are generally all approached aggressively, but are not killed immediately by the workers. We suggest that the distinctly controlled worker aggression towards gynes must be due to the fact that the gynes are sisters. Worker fitness will be increased by the departure of these sexuals, who are their sisters, because by flying out, the gynes may have reproductive success. According to this model, workers do benefit genetically from ‘chasing away’ their sister-gynes. In a following article (Sommeijer *et al*, 2003a) we supply evidence for the reproductive potential of non-accepted gynes that leave the nest.

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Samenvatting

Voortplantingsgedrag van angelloze bijen: verlaten van het nest en bruidsvluchten van *Melipona favosa* (Hymenoptera: Apidae, Meliponini).

Angelloze bijen (Apidae, Meliponini) zijn evenals de hongingbijen (Apidae, Apini) hoog-sociale bijen, dat wil zeggen met omvangrijke kolonies en complexe taakverdeling. Het gedrag binnen de kolonie verschilt sterk van dat van de honingbij (*Apis mellifera*). Dit geldt met name voor het voortplantingsgedrag. De soorten van het geslacht *Melipona* zijn binnen de angelloze bijen bijzonder door de afwijkende wijze van koninginnenproductie. Jonge koninginnen (‘gynes’) worden in grote aantallen en doorlopend geproduceerd. De tot nu toe beschreven voortplantingsmogelijkheden van deze jonge koninginnen zijn beperkt tot opvolging van de moederkoningin en vestiging in een dochterkolonie (door middel van ‘zwermen’). Echter juist bij *Melipona* komt vervanging van de oude koningin, evenals zwermgedrag, slechts sporadisch voor. Het agressief gedrag van werksters gericht op jonge koninginnen was al lang algemeen bekend; hierbij worden de jonge koninginnen gedood. Deze worden in de bestaande wetenschappelijke literatuur als overbodig aangeduid.

Ons doel was om te onderzoeken of de dagelijks geproduceerde jonge koninginnen mogelijk andere voortplantingsmogelijkheden hebben. Door gedetailleerde bestudering van het gedrag van jonge koninginnen in het nest en met een methode waarbij nestverlatende jonge koninginnen worden onderschept, konden wij vaststellen dat 57% ervan in staat is om uit te vliegen. Nadere analyse van het agressieve gedrag van de werksters laat zien dat deze agressieve interacties, die dagen kunnen duren, vaak voor langere tijd worden onderbroken. Werksteragressie leidt bijna nooit meteen tot de dood van de jonge koninginnen. Onze conclusie is dat juist door deze ‘gecontroleerde’ agressiviteit van de werksters jonge koninginnen er in kunnen slagen het nest te ontvluchten. Onze hypothese over de evolutionaire functie van dit gedrag is ontwikkeld in de lijn van gangbare theorie met betrekking tot de bijzondere verwantschappen binnen kolonies van de (haplodiploïde) sociale Hymenoptera: door de extreem hoge verwantschap van werksters met jonge zusterkoninginnen zijn de genen van de werksters erbij gebaat dat de jonge koninginnen uitvliegen om zich buiten de kolonie voort te planten. Een kernpunt bij deze hypothetische verklaring is de vraag of nestverlatende jonge koninginnen inderdaad de mogelijkheid hebben om tot voortplanting te komen. Dit wordt behandeld in een volgend artikel.