Reproductive behaviour of stingless bees: solitary gynes of *Melipona favosa* (Hymenoptera: Apidae, Meliponini) can penetrate existing nests

The stingless bee taxa of *Melipona* and *Trigona* differ fundamentally in their ways of producing new queens. *Trigona* stingless bees resemble honeybees (*Apis mellifera*) in that they rear new queens in special queen cells. In contrast *Melipona* stingless bees produce continually numerous queens from the standard type of brood cell. Earlier we found that 57% of the gynes of *Melipona favosa* are, in contrast to the general view, not killed by workers and are able to leave the nest if they are not accepted in the mother colony. In earlier observations we found that numbers of gynes visited a drone congregation site. While studying the extranidal behaviour of gynes we found that, on leaving the nest, these non-accepted gynes feed on flowers, and succeed in entering other *M. favosa* nests. These observations support our hypothesis that nest-departing non-accepted gynes of *Melipona* can contribute to reproduction in a way not reported previously. On basis of this result we are now able to propose a model of three alternative reproductive pathways for gynes. The fact that nest-departing non-accepted gynes engage in reproduction could explain the typical continuous production of large numbers of *Melipona* gynes. The establishment of new queens in colonies of all highly social bees has previously been reported to occur exclusively through 'swarming' or supersedure of the mother queen by a daughter queen. The reproduction strategies of *Melipona* appear to be unique within the social bees, since solitary new-born queens are able to penetrate existing colonies.

Entomologische Berichten 63 (2): 31-35

Marinus J. Sommeijer, Luc L.M. de Bruijn & Frans J.A.J. Meeuwsen

Department of Social Insects
Utrecht University
PO Box 80086
3508 TB Utrecht, The Netherlands
m.j.sommeijer@bio.uu.nl

Keywords: virgin queens, queen production, sexual behaviour, mating system

Introduction

The constant production of queens is a remarkable feature of the reproductive behaviour of all stingless-bee groups. The mass provisioning system for larval feeding in stingless bees does not allow for a prompt response to queen loss, and as a result replacement queens have to be available all the time. If there is queen loss in the nest of the honeybee (*Apis mellifera* Linnaeus), young worker larvae can be reared to become emergency queens by changing the larval diet. Generally, there are two systems for queen production in the stingless bees. In *Trigona* colonies, where queens are produced in large queen cells, only a few gynes are produced. Emerged *Trigona* gynes can be kept alive for a considerable time by the workers and in some species emerging queens are 'imprisoned' in empty food storage cells (Engels & Imperatriz-Fonseca 1990). In contrast, queen production in *Melipona* involves a continuous high production of gynes which all emerge from the regular one-sized brood cell (figure 1). Worker aggression towards gynes is very common in *Melipona* and gynes are seen to be killed by workers. Therefore, the production of gynes in *Melipona* stingless bees has always been considered as 'excessive', and all pre-

vious reports have claimed that all surplus gynes are killed. In a previous article on gyne behaviour we stated that this assumption needs to be reconsidered (Sommeijer *et al.* 2003). We established that a considerable proportion of these gynes are not eliminated by the workers but succeed in leaving the nest in good condition. We hypothesised that various other characteristics of *Melipona* worker behaviour serve as mechanisms facilitating the departure of gynes from the nest. Particularly important in this context is the fact that gynes are not killed immediately by the aggressively interacting workers. We explained that controlled worker aggression towards gynes has an evolutionary function because of the genetic relations within the colony. Gynes and workers are sisters and worker inclusive fitness (see text box 1) is increased by the departure of gynes, provided that departing gynes really can reproduce outside their maternal nest. Our study is based on the hypothesis that workers benefit genetically by chasing away their sister-gynes. The objective is to investigate whether nest-departing non-accepted gynes can reproduce outside the mother colony. The observations of which the results are reported here were carried out in Trinidad and Tobago, West-Indies, during fieldwork visits in the period 1993 to 2000.

Gynes visiting flowers

During our field studies of the flower-visiting behaviour of stingless bees and honeybees we observed visits made to flowers by gynes of *Melipona favosa* (Fabricius) (figure 2). In August 1993 they were seen foraging on three days, each time on flowers of corolita vine (*Antigonum leptopus*) and each time between 11.00 and 12.00 hrs. This tropical vine is an important nectar source, not only for stingless bees but also for honeybees and other bees in the neotropics. Photographs were taken of gynes imbibing the nectar of these flowers.

Gynes attracted by nest material

Gynes were seen flying around bee-stands containing nest boxes of *Melipona* and at other sites where *Melipona* hive material was handled. Gynes were attracted to nest boxes which had been emptied recently, and landed on parts of hollow trunks from which nests had been removed. They also visited isolated structures that had been taken out of nests, such as empty food pots, involucrum, 'batumen plates' and sheets of wax. Gynes landed on these materials but quickly flew away again. They were never seen to take up anything from these objects, not even honey that had sometimes been spilt. Such visiting gynes showed an active locomotive pattern, running fast with many turns and intensively rubbing their abdomen with their hind legs. While running, they often visited shady and dark crevices. Sometimes these gynes also flew to other contrasting dark objects that were only few metres away. During this behaviour the gynes had distinctly swollen abdomens. They appeared suddenly and we have no record of where they came from or where they went to next.

Figure 1. Horizontal brood combs of *Melipona trinitatis* from Trinidad W.I.. Workers, queens and males emerge from the same standard size brood cells.
Horizontale broedraten van Melipona trinitatis *uit Trinidad W.I.. Werksters, koninginnen en mannetjes komen uit hetzelfde standaardformaat broedcellen.*

Gynes entering existing nests

We observed the behaviour of sexuals and workers at drone congregation sites (figure 3; Sommeijer & de Bruijn 1995) and made additional observations during this study: we studied two drone congregation sites close to a nest of *M. favosa*. Based on flight intensity we concluded that these nests were in poor condition. On four occasions on three successive days (11-13 August 1998 between 11.00-12.00 hrs) we observed a gyne repeatedly trying to enter one of these nests. On two occasions gynes succeeded in getting past the guard. The first gyne entering the nest stayed inside for one and a half minute, the other stayed inside for at least five minutes. After a three-day observation period we examined the nests by opening them. In the first nest there were only fifteen recently emerged workers and one gyne which had emerged from her brood cell during our inspection. Both mature and young brood were present. There were 489

Box 1. Components of selection and fitness and the behaviour of social Hymenoptera

'Direct selection' acts on variation in individual reproductive success. 'Indirect selection' acts on variation in the effects individuals have on their relatives' reproductive success. 'Kin selection' is a form of natural selection where individuals differ in ways that affect their parental care or helping behaviour. (This influences the survival of their own offspring or of nondescendant kin.)

'Direct fitness' is measured in terms of personal reproductive ouput; 'indirect fitness' is measured in terms of genetic gains derived by helping relatives; 'inclusive fitness' is the sum of the two and represents the total genetic contribution of an individual to the subsequent generation.

Because of the haplodiploid nature of sex determination in the Hymenoptera (males only have one set of chromosomes and are called haploid, females have two sets of chromosomes and are called diploid), sisters may therefore be genetically very similar ($r = 0.75$) to one another, more so than a mother to her daughters and sons ($r = 0.50$). The essence of Hamilton's 'haplodiploid hypothesis' is that because of the genetics of sex determination in this group, worker hymenopterans can gain more 'inclusive fitness' by helping their reproductively competent sisters (future queens) than by reproducing themselves. (*After J. Alcock, 1998. Animal Behaviour, an Evolutionary Approach. Sixth edition, Sinauer Associates publ.*)

Figure 2. Gyne of *Melipona favosa* visiting flowers of the corolita vine *Antigonum leptopus*, foraging for nectar. Photo: L.L.M. de Bruijn
Maagdelijke koningin van Melipona favosa *foeragerend op bloemen van corolita* Antigonum leptopus.

cells of which 349 contained prepupae and pupae. The second nest also contained only a few young bees and resembled the first nest, except for the fact that it contained a considerable amount of stored food.

In a subsequent experiment we studied the nest-searching behaviour of gynes in more detail. We brought four wooden observation hives containing *M. favosa* nests to one of the drone congregation sites under observation and released eight individually marked gynes near the site. These gynes had been collected from emerging brood of other colonies in observation hives at a distance of ten kilometres. Immediately after release the gynes left the site and disappeared. However, after 30 minutes they all re-appeared, and while exhibiting searching behaviour they started to visit the entrances of the hives. The hives had been experimentally closed with wire mesh. The gynes also searched intensively among the dark folds of the black plastic and textile materials placed over the glass covers of these observation hives.

Discussion

The major question arising from the earlier observations of nest departures by non-accepted gynes (Sommeijer *et al.* 2003) is whether these gynes can truly reproduce outside the maternal nest. This question is difficult to answer directly. However, we can formulate several conditions that, in combination, supply circumstantial evidence to support the hypothesis that non-accepted nest-departing gynes become involved in reproduction. To do this successfully, the non-accepted nest-departing gynes should be able to:

a. leave the maternal nest in good condition,
b. stay alive for some time outside the maternal nest,
c. locate existing nests,
d. penetrate existing nests,
e. become accepted in a new nest,
f. make a nuptial flight.

ad a: in our previous report (Sommeijer *et al.* 2003) we found that 57% of a group of marked non-accepted gynes left their mother nest alive. Despite the fact that in the period before leaving these gynes had been attacked by workers, a number of gynes managed to escape;

ad b: we now have evidence that gynes are able to feed themselves in the field. Our observations have shown that they visit flowers of the vine *Antigonum leptopus*, where they imbibe nectar. Extranidal feeding may be restricted to nectar intake from flowers; during our frequent observations of gynes visiting abandoned nest material including honey deposits, we never saw gynes feeding from these sources;

ad c: the fact that gynes visit exposed nest material including empty hives, land at drone congregation sites, and are attracted by crevices and nest exits, provides evidence that in this behavioural phase gynes search for existing nests;

ad d: on two occasions we saw searching gynes actually entering existing nests. These were gynes that had visited drone congregation sites close to nests. The interactions of the gyne with the guard at the nest entrance of these declining colonies indicated that entering was not easy. However, the persistence of the gynes' attempts to enter the nest shows that gynes have a strong drive to enter existing nests. This was also confirmed by the behaviour of gynes released near a drone congregation where four nest boxes containing *M. favosa* colonies had been temporarily installed;

ad e: as yet, we cannot supply evidence for the acceptance of gynes in a new nest. However, the fact that queenless workers of *Melipona* take a long time to finally accept one of their own sister-gynes (Silva *et al.* 1972, Van Veen *et al.* 1999) means that it should be possible for gynes from other colonies to penetrate and become accepted during that period. One indication of 'foreign' gynes entering the nest may be accepted is that laying queens can easily be exchanged between experimental *Melipona* colonies (M.J. Sommeijer personal observation). The two nests penetrated by the gynes contained only a few workers but a considerable amount of brood. For a successful take-over of nests it is probably important that part of the brood is mature. The presence of only a limited number of workers may make the nest even more suitable for take-over, since there will be little risk of aggressive workers;

ad f: so far we have not been able to assess whether invading gynes are already inseminated. Theoretically, gynes can become inseminated during different phases in this behavioural system: 1 - immediately after they leave the maternal

Figure 3. Drone congregation site of *Melipona favosa*. Hundreds of males are congregating on a vertical substrate.
Darrenverzamelplaats van Melipona favosa. *Een groep van honderden mannetjes zit bij elkaar op een verticale ondergrond.*

nest, 2 - at the stage when they are searching for other existing nests, or 3 - after acceptance in the new nest. The fact that a large number of gynes were observed at a drone congregation site during a very short period of time (Sommeijer & de Bruijn 1995) rules out the possibility that gynes are inseminated after being accepted in a new nest. After all, we cannot expect a large number of colonies within a small area and over a very short time to be in the phase of queen supersedure or swarming. It is important to find out at what moment insemination occurs during this behavioural pathway.

A new model of the reproductive destinies of gynes

Figure 4, which integrates the results on this topic, depicts a preliminary model of the various reproductive destinies of gynes of *Melipona*. When a gyne is born she can follow four different pathways: 1 - while still in the nest she may be killed by workers, 2 - she may swarm with a part of the colony to establish a new nest, 3 - she may become involved in reproduction if she is able to supersede her mother in the old nest, 4 - non-accepted gynes leave the nest and may reproduce after infiltrating an other nest. We conclude that the present results supply evidence for this latter hitherto unknown pathway for reproduction: gynes are able to leave the maternal nest if not accepted (Sommeijer *et al.* 2003) and may subsequently take over an existing nest.

The evolutionary function of differences between Melipona and Trigona

Since our model depicts a new way of investigating the evolutionary function of the large numbers of *Melipona* gynes at any given time, it is important to compare *Melipona* bees with *Trigona* bees. In relation to an evolutionary function of the production of large numbers of *Melipona* gynes at any given time, which does not occur in the group of *Trigona,* it can be hypothesised that *Trigona* colonies have a higher mortality rate, since they nest in more dynamic environments and in less stable substrates. This behaviour of *Trigona* stingless bees may be comparable to the frequent swarming of certain African subspecies of *A. mellifera*. *Melipona* nests generally exist for long periods and *Melipona* queens live for a long time. It is known that certain *Trigona* species regularly produce daughter colonies (Engels & Imperatriz-Fonseca 1990, Van Veen *et al.* 2000), but there have been very few observations of typical 'swarming' behaviour in *Melipona*. The ecological function of variations within the group of *Trigona* bees will have to be studied in the light of the present results. More detailed observational studies and the use of new genetic methodology (DNA analysis) are needed to further clarify the evolutionary questions relating to the study of reproduction in stingless bees.

Melipona occupies a unique position among the social bees

In his comprehensive treatment of the systematics of the bees of the world, Michener (2000) discusses the basal position of *Melipona* in the cladogram based on his earlier work (Michener 1990). Based on our behavioural observations, *Melipona* bees can be said to occupy a unique position in relation to colony reproduction if nest-departing gynes in *Trigona* colonies do not exist. Colony foundation has been reported to occur in all highly social bees through colony di-

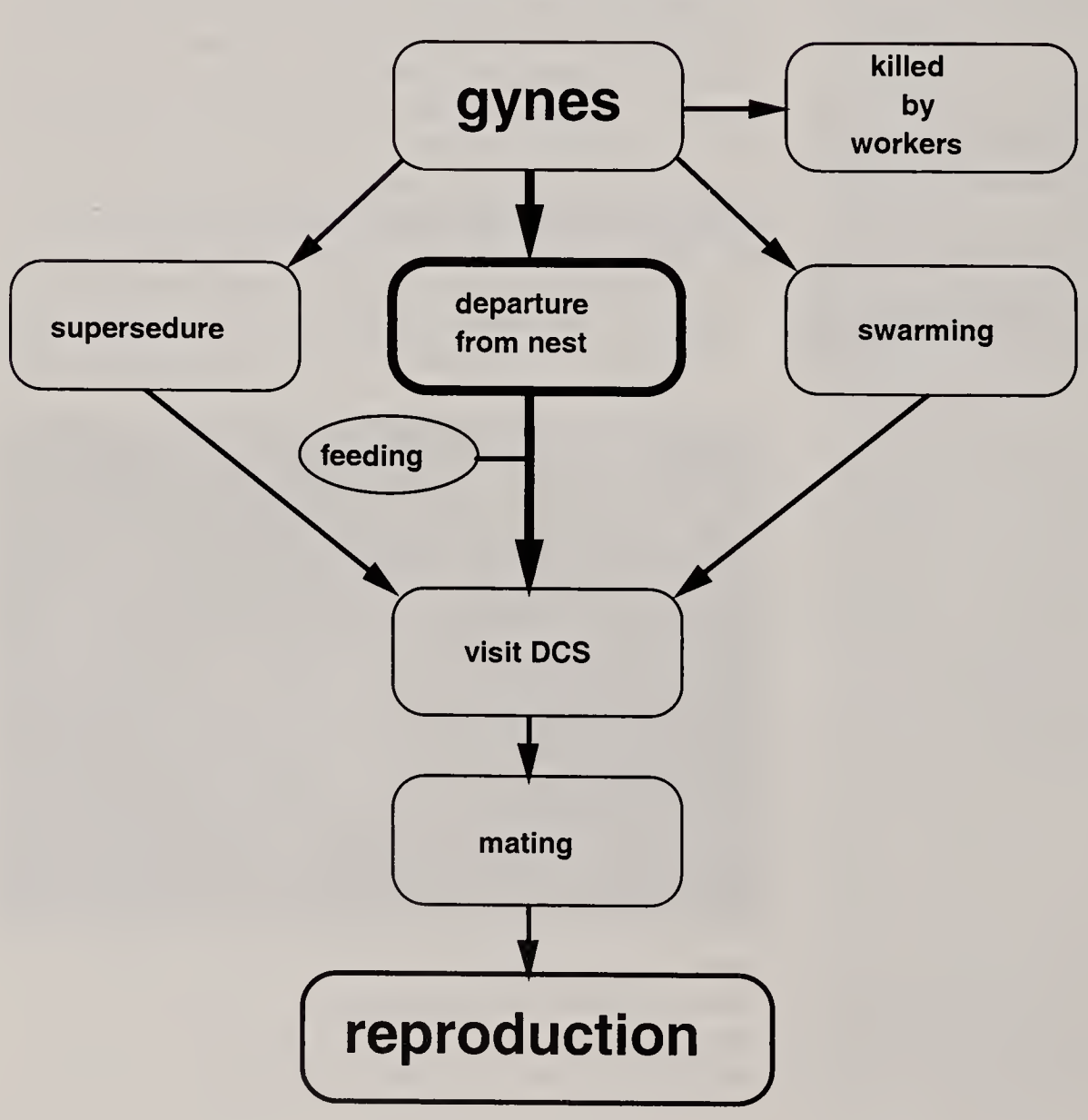

Figure 4. Model of reproductive pathways of *Melipona* gynes. When a gyne is born, she can follow four different pathways: 1 - she may be killed by workers, 2 - she may swarm with a part of the colony to establish a new nest, 3 - she may become reproductive when she is able to supersede her mother in the old nest, 4 - non-accepted gynes depart from the nest and may succeed in becoming reproductive by penetrating another nest. DCS = drone congregation site.
Model van manieren van voortplanting van maagdelijke koninginnen ('gynes') bij Melipona. *Na het uitkomen kunnen maagdelijke koninginnen vier verschillende routes volgen: 1 - ze kan gedood worden door werksters, 2 - ze kan meezwermen tijdens afsplitsing van een dochternest, 3 - ze kan in het nest de oude koningin vervangen, 4 - ze kan het nest verlaten en zich buiten het eigen nest voortplanten door een ander nest binnen te dringen. DCS = darrenverzamelplaats.*

vision ('swarming'). The establishment of new queens of highly social bees has been reported to occur by 'swarming' of their own nest or by supersedure of their mother. Since new-born queens in *Melipona* are able to penetrate existing colonies solitarily, *Melipona* reproductive behaviour differs fundamentally from the reproductive strategies practised by other highly social bees.

Acknowledgements

The first author wishes to thank all of the MSc and PhD students who participated in this research programme on stingless bees. The enthusiasm of these students, whether they were involved in laboratory studies at Utrecht University or in field projects in the tropics, has always been valuable and stimulating. The authors are grateful to the management of Burgers' Zoo, Arnhem, The Netherlands, for allowing them to carry out research projects in Burgers' Bush. They thank the friendly collaborators in the tropics, especially in Costa Rica and in Trinidad and Tobago W.I., for their valuable contribution. The second author is grateful to the Uyttenboogaart-Eliasen Stichting for supporting his work on Trinidad and Tobago. Jacques van Alphen and Theo Peeters are thanked for comments on an earlier version of this paper. Sheila McNab is thanked for linguistic advice.

References

Chinh TX, Grob GBJ, Meeuwsen FJAJ & Sommeijer MJ 2003. Patterns of male production in the stingless bee *Melipona favosa* (Apidae, Meliponini). Apidologie 50 (1-2) (in press).

Engels W & Imperatriz-Fonseca VL 1990. Caste development, reproductive strategies, and control of fertility in honey bees and stingless bees. In: Social insects, an evolutionary approach to castes and reproduction (Engels W ed.): 167-230. Springer Verlag.

Michener CD 1990. Classification of the Apidae. University of Kansas Science Bulletin 54: 75-153.

Michener CD 2000. The bees of the world. Johns Hopkins University Press.

Silva DL Naves da, Zucchi R & Kerr WE 1972. Biological and behavioural aspects of the reproduction in some species of *Melipona* (Hymenoptera, Apidae, Meliponinae). Animal Behaviour 20: 123-132.

Sommeijer MJ & Bruijn LLM de 1994. Intranidal feeding, trophallaxis and sociality in stingless bees. In: Nourishment and Evolution in Insect Societies. (Hunt J & Nalepa C ed.): 391-418. Westview Press.

Sommeijer MJ & Bruijn LLM de 1995. Drone congregations apart from the nest in *Melipona favosa*. Insectes Sociaux 42: 123-127.

Sommeijer MJ, Bruijn LLM de, Meeuwsen FJAJ & Slaa EJ 2003. Reproductive behaviour of stingless bees: nuptial flights and nest departing non-accepted gynes in *Melipona favosa*. Entomologische Berichten 63: 7-13.

Veen JW van & Sommeijer MJ 2000. Colony reproduction in *Tetragonisca angustula* (Apidae, Meliponini). Insectes Sociaux 47: 70-75.

Veen JW van, Sommeijer MJ & Aguilar Monge I 1999. Behavioural development and abdomen inflation of gynes and newly mated queens of *Melipona beecheii* (Apidae, Meliponinae). Insectes sociaux 46: 361-365.

Accepted 20 January 2003.

Samenvatting

Voortplantingsgedrag van angelloze bijen: solitaire maagdelijke koninginnen van *Melipona favosa* (Hymenoptera: Apidae, Meliponini) kunnen bestaande nesten binnendringen

Angelloze bijen van het geslacht *Melipona* produceren grote aantallen jonge maagdelijke koninginnen ('gynes') die maar zelden tot vervanging van de moederkoningin of tot vestiging van een dochterkolonie kunnen komen. In een voorgaand onderzoek vonden wij dat 57% van deze maagdelijke koninginnen in staat was om de kolonie te verlaten en zo te ontsnappen aan werksteragressie. Het huidige onderzoek ondersteunt met waarnemingen aan uitvliegende maagdelijke koninginnen de hypothese dat deze nestverlatende bijen de mogelijkheid hebben om zich voort te planten. Eerder vonden we dat maagdelijke koninginnen op darrenverzamelplaatsen in grotere aantallen zijn waargenomen dan verwacht kon worden op grond van veronderstelde aantallen kolonies in de omgeving waar de koningin vervangen wordt of waar een zwerm wordt afgesplitst. Maagdelijke koninginnen zijn nu ook foeragerend waargenomen op bloemen en rondvliegend bij nesten op bijenstanden met angelloze bijen. Een sterke ondersteuning voor de hypothese dat maagdelijke koninginnen zich buiten het moedernest kunnen voortplanten wordt geleverd door de waarneming van maagdelijke koninginnen die bestaande nesten binnendringen. Door een experiment waarbij in kasten gehuisveste kolonies bij een darrenverzamelplaats werden geplaatst en vervolgens maagdelijke koninginnen werden losgelaten werd bevestigd dat maagdelijke koninginnen bestaande nesten opzoeken en hier naar binnen proberen te gaan. Wij concluderen dat de bestaande opvatting over de reproductieve betekenis van de tot voor kort als 'overbodige' grote aantallen maagdelijke koninginnen in *Melipona*-nesten moet worden bijgesteld. De haplodiploïdie van Hymenoptera, welke een buitengewoon grote verwantschap tussen vrouwelijke nakomelingen van de koningin oplevert, is de basis voor theorievorming over evolutie van typische gedragingen bij kolonievormende Hymenoptera. De opvallende maagdelijke koningin productie en het agressief gedrag van *Melipona* werksters kan nu ook vanuit deze theorie verklaard worden. De mogelijkheid van nestverlatende *Melipona* maagdelijke koninginnen om zich buiten het nest voort te planten, blijkt het belang voor de verspreiding van de genen van werksters te dienen (verhoogt de 'inclusive fitness' van werksters). Tot voor kort was er bekend dat koninginnen van hoog sociale bijen zich alleen konden voortplanten door zwermen en door vervanging van de oude koningin in het nest. Door deze resultaten is het duidelijk dat het geslacht *Melipona* een hiervan afwijkende voortplantingsstrategie bezit.