Chapter 6
Reproduction

In collaboration with Teresita Solís

Mating in *M. beecheii*

There are two ways in which social insects can reproduce: one is through the production of sexual offspring by queens, males, and (in some species) workers, and the other is through colony fission (Ruttner 1985; Koeniger 1986). This means that, in social insects, effective reproduction depends not only on individual performance, but also to a large extent on the functioning of the colony. Both aspects are tightly linked; survival of the colony is in the best interests of all colony members to ensure high output of reproductives and daughter colonies (Crozier and Pamilo 2003).

Sexual offspring in stingless bees can be produced by queens and males, but also workers. As explained in Chap. 4, the workers of many species are capable of pro-

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ducing male offspring, though whether the queen or workers dominate in male production varies considerably between species, and also between colonies within a species (Paxton et al. 2001; Tóth et al. 2002). Because the males of stingless bees live a solitary life when reaching sexual maturity, they seem a relatively "cheap" way of genetic dispersal, although male production generally occurs only when colonies have accumulated sufficiently large food reserves (Moo-Valle et al. 2001). In *Melipona*, gynes also invade colonies (Sommeijer et al. 2003), which may represent another type of "cheap" genetic dispersal, via females. Nonetheless, the importance of gyne invasion of non-related colonies in natural populations remains unknown. The same applies to workers; although workers can potentially parasitize other colonies, no evidence of this has been found.

In addition to individual reproduction, propagation in social insects through the female line, involves the production of workers that depart with the queen in a swarm (Wilson 1971). Notably, reproduction of colonies in the highly eusocial bees (meliponines and honey bees) invariably occurs through colony fission (splitting) (Michener 1974; Hölldobler and Wilson 1990). The mother colony divides into two (and exceptionally more) colonies, each with a group of workers and generally one queen. Thus, the investment in colony reproduction through swarming is large compared to the investment in the production of individual sexual offspring (Fig. 6.1).

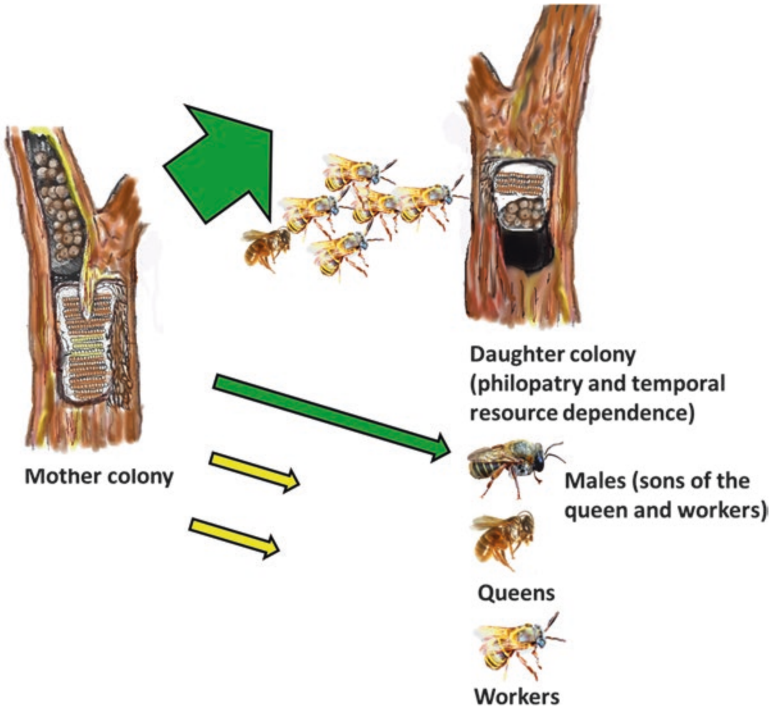


Fig. 6.1 Reproductive investment and potential routes of genetic dispersal in Meliponini. The thickness of the arrow represents the magnitude of resource investment, and its length the potential distance dispersal. Female dispersal via queens and workers has not been well documented in non-managed populations

In addition, colony reproduction seems especially demanding in the case of stingless bees because daughter colonies are not completely independent after departing. There is a long-lasting bond between mother and daughter colony until the latter is self-sufficient (Engels and Imperatriz-Fonseca 1990). This also implies that in stingless bees dispersal via females involves comparatively shorter distances (philopatry), than via males.

Although highly eusocial bees share some common features in the reproduction of colonies and individuals, there are also marked contrasts in their reproductive biology which are presented in Table 6.1.

Table 6.1 Comparison of some reproductive traits between stingless bees and honey bees

Individual traits	Stingless bees	Honey bees
Sexual dimorphism	Males larger than gynes in *Melipona* Males smaller than gynes in non-*Melipona*	Males larger than gynes in *Apis mellifera* but smaller in other *Apis* species
Virgin queen	At emergence, ovaries well developed in non-*Melipona* gynes and underdeveloped in *Melipona* Ovarioles have increased in size rather than number (usually $n = 4$) Queens tolerate each other Workers eliminate excess queens	At emergence ovaries well developed Ovarioles have increased in number rather than length ($n > 100$) Queens do not tolerate each other Queens eliminate other queens
Males	Sexually immature at emergence Solitary lifestyle when sexual maturity is reached	Sexually immature at emergence Live in colonies when sexual maturity is reached
Reproductive workers	Frequent in the presence of a queen (queen's chemical control of ovarian development rare). No worker policing has been documented	Rare (queen's chemical control of ovarian development). Worker policing exists
Queen mating	Species predominantly monandrous In non-*Melipona*, males congregate nearby colonies searching for virgin queens Males of *Melipona* possibly congregate far away from colonies and mating seems to occur in these areas	All species are polyandrous Males congregate far away from colonies
Male mating	Monogynous—males die after mating	Monogynous—males die after mating
Mated queen	Physogastric—unable to fly	Non-physogastric—capable of flying
Contact workers-queen	Workers have little contact with the queen—the queen is frequently aggressive towards workers Ritualized oviposition	Workers frequently groom the queen; there is a queen retinue—no evident physical aggression of the queen Non-ritualized oviposition
Queen feeding	Mainly trophic eggs produced by the workers	Royal jelly produced in glands in the head of workers
Colony traits		
Composition of swarm	Non-mated gynes and workers	Mother queen and workers

(continued)

Table 6.1 (continued)

Individual traits	Stingless bees	Honey bees
After swarming	Non existent	Frequent in some populations
Contact between mother and daughter colony	Daughter colony located nearby Long-lasting contact with mother colony	Daughter colony moves far away No further contact between mother and daughter colonies
Preparation of the new nest	Anticipated	No preparation before swarming
Reproductive strategy	Few offspring colonies with high survival rates ("K" strategy)	Tropical ecotypes: more offspring colonies with relatively low survival rates ("R" strategy)

6.1 Individual Reproduction

The production and behavior of sexual offspringin stingless bees show important differences with the honey bees (Table 6.1).

When completing their development, queens of stingless bees can emerge rapidly from their cell, as in *Melipona,* or prolonged, sometimes needing the help of workers, like in most other genera. Moreover, the queens of genera different to *Melipona* seem to take longer to develop (pers. Obs.), in comparison with the queens of *Melipona* (Moo-Valle et al. 2004). Possibly, these differences in duration of development can explain why *Melipona* gynes emerge with underdeveloped ovaries, compared with other genera of stingless bees (Wille 1983).

In the meliponines, the behavior of workers towards queens varies across species. In most non-*Melipona* species, gynes at emergence are generally not attacked by workers (Gutiérrez et al. 2016). In contrast, in *Melipona*, workers are frequently aggressive to gynes; indeed, most excess queens are rapidly killed in the colonies (Wenseleers et al. 2004; Jarau et al. 2009; Kärcher et al. 2013). On the other hand, stingless bee queens are rather indifferent to each other. There is no aggression among gynes or with the mother queen (Kärcher et al. 2013). In comparison, honey bee queens are not tolerant to other queens; they fight each other to death (Winston 1987).

After the queen emerges she can be kept alive for some time. Some non-*Melipona* species build special "compartments" where they maintain gynes, these could be empty food pots or spaces in the involucrum. Such lodgings are known as royal chambers and may serve to keep gynes as insurance in case of the loss of the mother queen. Imperatriz-Fonseca and Zucchi (1995) report that in some species, the gyne participates in the construction of her own chamber and can even control the access of workers (e.g., in *Frieseomelitta*, *Plebeia*). In other species like *Cel. longicornis*, a few workers form a shield around gynes to protect them against attacks by other workers (Imperatriz-Fonseca and Zucchi 1995). Because swarming rates in stingless bees are low, most queens are eventually sacrificed.

Melipona species are outstanding for the excess number of queens produced (see Chap. 4). However, most queens are killed (Wenseleers et al. 2004; Kärcher et al. 2013). In *M. beecheii,* for instance, gynes live on average only 27 h (Wenseleers

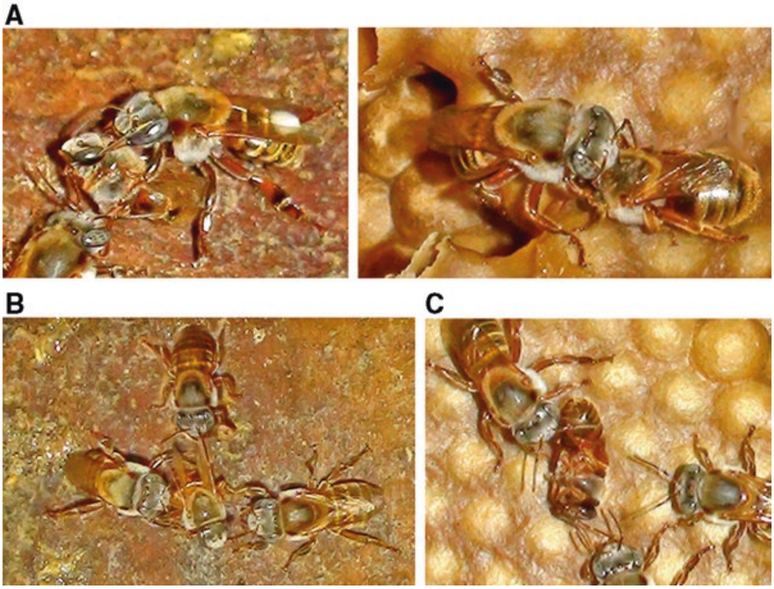


Fig. 6.2 The process of gyne sacrifice in *M. beecheii*. (**a**) Two aspects of decapitation by a single worker. (**b**) Pulling extremities by various workers. (**c**) A gyne faking death to avoid worker aggression

et al. 2004). In this species, two forms of queen sacrifice have been recorded (Fig. 6.2). Approximately 25% of sacrifices are made by a single worker. She climbs on the gyne's dorsum and, inserting the mandibles in the space between the head and the thorax, quickly proceeds to decapitate her. In a second and more frequent behavior, a group of workers hold the queen and pull her from the legs, head, and abdomen, trying to dismember her (Fig. 6.2). This last type of killing can last up to 1 h. In many cases, gynes try to hide from the workers, and can even fake death to avoid elimination (Fig. 6.2, Wenseleers et al. 2004). It seems that less "fit" queens fake death more frequently, as those engaging in this type of behavior are eventually killed (van Veen et al. 1999). If queens avoid immediate killing, they can reach a stage when start performing a behavior called abdominal inflation. Abdominal inflation start in *M. favosa* and *M. beecheii* some 12–15 h after gyne emergence and becomes more frequent with time (Koedam et al. 1995; van Veen et al. 1999). In *M. beecheii* all queens eventually accepted in colonies performed abdominal inflation. In some way queens with an inflated abdomen could be more attractive to workers (van Veen et al. 1999). Gynes in *Scaptotrigona* inflate the abdomen and use it to hit the workers that try to bite them. Perhaps this behavior is used to defend from worker aggression and is also part of a process of queen domination (Gutiérrez et al. 2016). Abdominal inflation could also be related with pheromone production (van Veen et al. 1999), but this has not been demonstrated.

The excess production of gynes in *Melipona* and aggressive harassment by their worker sisters, are possibly the origin of a phenomenon observed in meliponaries, i.e., the invasion of unrelated colonies by “runaway” gynes (Sommeijer et al. 2003). This behavior was first observed in the Caribbean species *M. favosa,* but no evidence of reproductive takeover could be confirmed. The authors interpreted the aggression of the workers as another way by which colonies (and workers) could increase reproductive fitness via dispersal of their gyne sisters, if these were to become queens in invaded colonies (Sommeijer et al. 2003) (Fig. 6.1). The rate of gyne colony usurp was quantified by means of molecular analyses in *M. scutellaris* from Brazil. Gynes of this species exhibited surprisingly high rates of colony invasion estimated in ~25% (Wenseleers et al. 2011). Interestingly, gynes seem to preferentially take over queenless colonies at night, which suggests that this behavior does not occur randomly (van Oystaeyen et al. 2013). Given the high frequency at which queen usurping could occur, it is puzzling why colonies do not seem to have developed mechanisms to reject alien queens (Kärcher et al. 2013; van Oystaeyen et al. 2013).

In *M. beecheii* we have observed (unpublished data) that many gynes exit their colonies and seek refuge in areas like empty boxes or under pieces of wood. However, most of these gynes do not survive long, probably because they need to be fed by workers, although it has been suggested that they can probably feed on flowers (Sommeijer and de Bruijn 2003). Marking large number of *M. beecheii* gynes has failed to detect them in other colonies, but molecular analysis has not yet been conducted to confirm if gyne colony takeover could be common in this species. Although gyne usurping seems frequent in meliponarios, there is still no evidence of its occurrence in non-managed populations (Wenseleers et al. 2011) and, thus, whether it represents a significant mode of female gene dispersal in natural populations of stingless bees.

Female reproductive parasitism in stingless bees can potentially occur via workers too. Workers, similar to gynes, can abandon their natal nest and invade other colonies, where they can produce sons (Beekman and Oldroyd 2008). To date no such phenomenon has been documented in any species of stingless bee. However, worker parasitism across generations has been detected. In *M. scutellaris* workers carry on producing sons, even after their mother queen has been superseded. In this sense, daughters of the old queen parasite workers of the new generation (their nieces), with which they are less genetically related (Alves et al. 2009).

Probably because of the large number of gynes produced in *Melipona*, colonies seem highly selective in the process of queen replacement. In a study to evaluate the process of queen acceptance in *M. beecheii*, it was found that colonies can remain queenless for up to 3 weeks and can kill as many as 20 queens in this period (Flores-Grande et al. unpublished data). Similar results have been obtained with other species, like Brazilian *M. compressipes* (Kerr 1987). Even though in *Melipona* there is strong selection on queens, the role of factors such as genotype, aggression, and chemical profiles, among others, is still not well understood (Kerr 1987; Gutiérrez et al. 2016). The speed of queen acceptance has important applications in management because, if the queen is lost, the colony may remain queenless for several weeks, which can affect growth and possibly compromise its survival.

After selection, the accepted gyne experiences notorious changes in behavior before her mating flight. In this period, the gyne increases her activity, jostling and performing considerable antennal contact and trophallaxis or food exchange with workers (van

Veen 1999). Aggression towards workers, like pushing or jostling, could be considered part of a gradual process of domination. In fact, physogastric queens frequently push and hit the workers during the POP (Sakagami 1982; Jarau et al. 2009).

Regarding queen mating, Flores-Grande et al. (2003) found that gynes of *M. beecheii* made a single mating flight. On the day of the mating flight, the gyne exits the colony and makes a short "orientation" flight. She performs a first flight facing the entrance of her colony in a zigzag pattern, possibly registering landmarks to help in finding the colony. Slowly, the gyne flies away from the colony until she rapidly disappears into the forest. The age at which gynes of *M. beecheii* undertook their mating flights was 4.6 days in average, and the duration of the flight was only 10.8 min. In studies with *M. beecheii* in Costa Rica, the duration of mating flights was over 100 min (van Veen and Sommeijer 2000). It is possible that such differences in mating flights for the same species relate to the availability of males in both areas, and the possibility to find them nearby. Mating flights took place more frequently at midday, between 11:30 and 12:50 h. The average temperature registered for the mating flights in Yucatan was 31.1 °C, the environmental humidity was 38.2%, and wind speed was 0.58 m/s. The average age at which queens started egg laying was 10.4 days after emergence, and 5.8 days after their mating flight (Flores-Grande et al. 2003).

As a norm, stingless bees queens are monandrous, they mate with only one male (Peters et al. 1999; Paxton et al. 1999; Palmer et al. 2002). After mating, the genital capsule of the male detaches from his body and remains inserted in the queen's reproductive tract for a couple of days, forming what is known as the mating plug (Melo et al. 2001) (Fig. 6.3). It is thought that the male capsule causes distension of

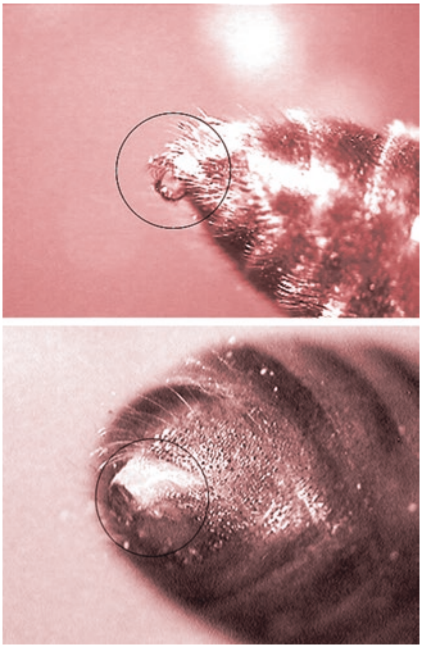


Fig. 6.3 Mating plug formed by the genital capsule of the male in the reproductive tract of a queen of *M. beecheii* (photo by Fredy Camposeco)

the *bursa copulatrix* of the queen, and produces a mechanical signal. This signal may be related to the activation of the *corpora allata,* and the production of JH that triggers ovary growth. In a few *M. quadrifasciata* queens, removing the male genital capsule in the first 3 days after mating resulted in reduced activation of the queen's ovaries (Melo et al. 2001).

In contrast with males of honey bees and bumble bees, the mating plug of stingless bee males has no glandular products. Thus, it is believed that there is no male chemical stimulation that may induce ovarian development in stingless bee queens, as known in the other corbiculate taxa (Colonello and Harfelder 2005).

After the mating flight, the queen reduces food soliciting, and her activity within the nest markedly decreases, as does aggression towards workers (van Veen 1999). At the same time the abdomen experiences a remarkable increase in size, known as physogastria. Physogastric queens (also seen in termites and some ants) experience a dramatic hypertrophy of the ovaries. In the process, the fat bodies in the abdomen also experience considerable growth. The fat bodies are the main source of vitellogenin, a major lipoprotein released into the hemolymph and taken up by the ovarioles for the production of eggs (Bordereau 1982; Paes de Oliveira and Cruz-Landim 2003; Paes de Oliveira et al. 2012).

Physogastria produces extreme distension of the abdomen, causing the intersegmental membranes of the metasoma to expand (Bordereau 1982). The abdominal terga and sterna become well separated in stingless bee queens, which does not occur in honey bee or bumble bee queens (Fig. 6.4). As a result of the physogastric state, stingless bee queens can double their weight and become incapable of flying.

The females, (workers and queens) of stingless bees, normally have four ovarioles in each of their two ovaries (Cruz-Landim et al. 1998; Martins and Serrâo 2004). The workers' ovaries are comparatively shorter than the queens' (Cruz-Landim 2000) (Figs. 6.4 and 6.5). In a few species (*Tr. spinipes, Pb. remota, N. testaceicornis, Sch. quadripunctata*), the queens can have up to eight ovarioles per ovary (Cruz-Landim et al. 1998), but in *Lestrimelitta,* the number of ovarioles per ovary can be up to 15 (Sakagami 1982). Nonetheless, the number of ovarioles per ovary in stingless bees is in stark contrast to the more than 100 present in honey bee queens (Engels and Imperatriz-Fonseca 1990; Martins and Serrâo 2004).

It is suggested that the high demand for egg production in queens of the highly eusocial bees was solved through different evolutionary routes. In Meliponini, queens experienced an increase in the relative length of the ovaries more than an increase in number. In contrast, queens of *Apis* have experienced an increase in the number of ovarioles, rather than in longitude (Engels and Imperatriz-Fonseca 1990; Martins and Serrâo 2004).

Intraspecific variation in the length (and less frequently number) of ovarioles in stingless bee queens has been observed. This variation may be related to differences in nutrition during gyne development. In *Tr. spinipes* larvae fed with larger amounts of food had more and longer ovarioles (Buschini and Campos 1995; Lisboa et al. 2005).

In stingless bee queens, each ovary usually produces between one and a few dozen per day, depending on the species (Engels and Imperatriz-Fonseca 1990). The rate of queen egg production per day has been recorded in some species: in

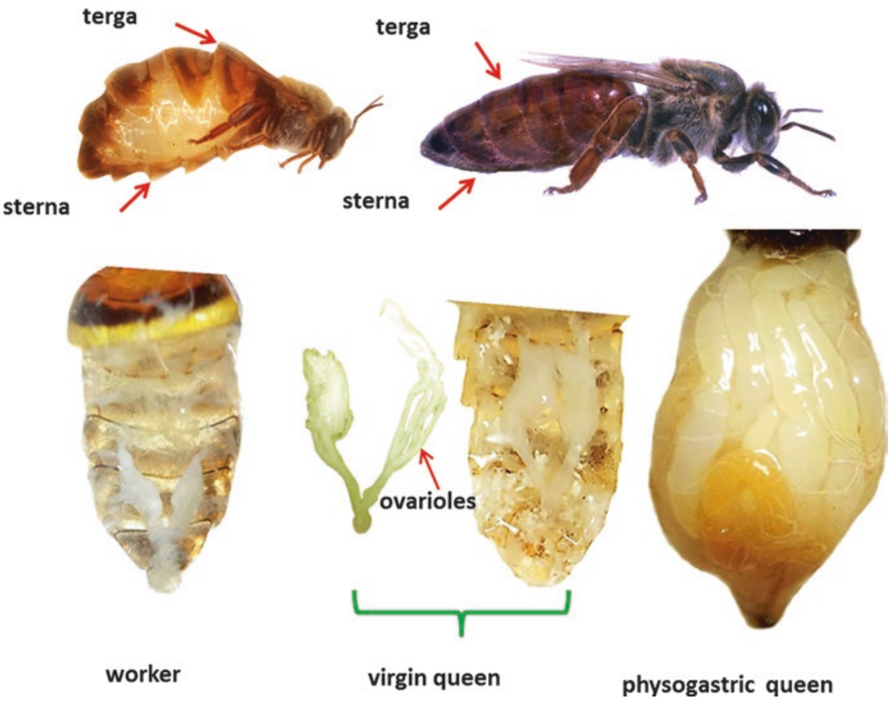


Fig. 6.4 Physogastric queen of *M. beecheii* (above left), showing the distended abdomen with clearly visible abdominal membranes and separated plates compared with *A. mellifera*, in which queens are non-physogastric. In the image below, the ovaries of worker, virgin (with the four ovarioles separated), and physogastric queen of *M. beecheii,* are shown

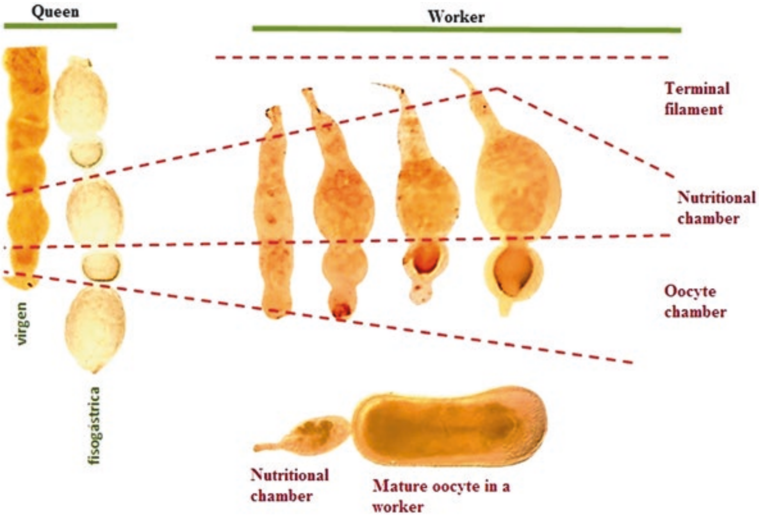


Fig. 6.5 Oogenesis in a worker and the queen of *M. beecheii*, with the parts of the ovarian follicle: the oocyte chamber and the nutritional chamber. The image below shows an egg nearly ready to be laid; the nutritional chamber is atrophied

M. compressipes it is between 25 and 30 eggs, in *M. quadrifasciata* between 10 and 20 (Kerr 1949), in *P. remota* between 60 and 180 (Van Benthem et al. 1995), in *M. beecheii* between 25 and 35, and in *L. niitkinb,* between 100 and 200 eggs per day (pers. Obs.). The rate of egg production of queens seem to depend on colony food conditions (Moo-Valle et al. 2001).

The production of eggs in the ovaries of queens (and workers) starts in the ovarian functional unit, the follicle. The ovarian follicle is formed by two sections, the nutritional chamber and the oocyte chamber (Fig. 6.5). The nutritional chamber is the section that supplies nutrients to the oocyte by means of special cells called trophocytes. The trophocytes provide vitellogenin and other proteins needed for the development of the oocyte (Cruz-Landim 2009). In the process of oogenesis, as nutrients are absorbed by the oocyte chamber, the nutritional chamber experiences gradual atrophy. The final stages of egg formation involve the production of the vitelline membrane as well as the chorion, forming the egg shell (Cruz-Landim 2009). The chorion of the bee's egg has two layers, a proteinic endochorion and the exochorion mainly made of carbohydrate (Cruz-Landim 2009).

The ovaries of the queen have several follicles undergoing maturation at the same time; in contrast, the ovaries of workers only have one maturing follicle, and exceptionally two, in each ovary (Cruz-Landim 2000; Figs. 6.4 and 6.5).

The rate of ovarian development among workers of the same colony generally depends on age. Young workers less than 1 week old usually have no developed ovaries, but after this period, they start producing trophic or reproductively functional eggs. The production of reproductive eggs is more frequent in workers before they start foraging (van Veen 1999). In older workers (foragers), the ovaries usually become atrophied (Fig. 6.6). Functional worker eggs are not fertilized, but develop

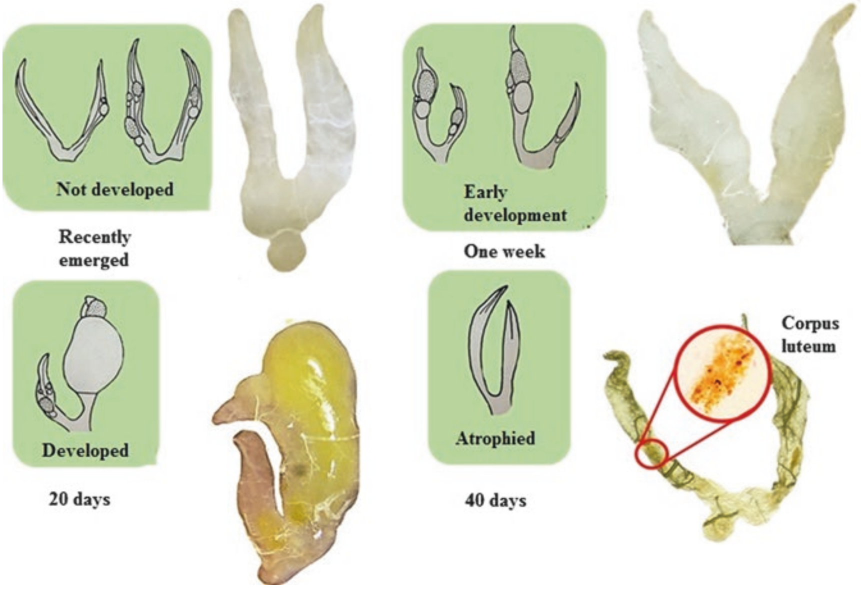


Fig. 6.6 Comparative development of the ovaries in workers of different age in *M. beecheii*

into males by parthenogenesis, similar to those of the queen (Koedam et al. 1999). The characteristics of trophic and functional eggs in stingless bees are explained in more detail in Sect. 5.3.

An outstanding fact of mated queens of social insects is the extremely long viability of the sperm they store, which may span several years (Baer et al. 2016). It is believed that the viability of sperm is maintained thanks to the stable conditions of the spermatheca. The spermatheca is a spherical sac at the base of the queen's oviducts where sperm is stored after mating (Cruz-Landim et al. 2003). To date it is not quite understood how the viability of the sperm is maintained in the spermatheca. Honey bee queens may actively produce proteins to enhance sperm viability during storage (den Boer et al. 2009). Interestingly, compounds that keep the sperm alive may not be produced in the spermatheca; they could be absorbed from the hemolymph, and secreted around the stored sperm (Cruz-Landim et al. 2003). The spermatheca is connected to the reproductive tract by a small tube in the transition between the *bursa copulatrix* and the midportion of the oviduct (Fig. 6.4).

Hymenopteran queens have control over sperm use by means of the spermatheca, and can switch between producing fertilized and non-fertilized eggs. In the honey bee, failure of the queen to fertilize worker-destined eggs seems to be a signal that workers use to replace her (Baer et al. 2016). In stingless bees, the longevity of mated queens and the causes leading to supersedure are scarcely documented. In queens of *M. compressipes* and *M. scutellaris*, a maximum longevity of 7 years has been estimated (Kerr 1996; Kerr et al. 1996), although a more conservative figure of 22 months could be more realistic (Carvalho-Zilse and Kerr 2004). Similarly, in *M. beecheii* queens more often seem to live an average of 2 years (van Veen 1999).

The males of stingless bees can be produced by the queen or the workers. As explained in Chap. 4, the relative numbers of sons of the queen and workers vary across species. In species in which males are mostly produced by the workers, the cells containing males tend to form clusters in some sections of the combs (Koedam et al. 1999). In the case of species like *M. beecheii* (Paxton et al. 2001) and *M. colimana,* in which the queens produce most of the males, there is no clustering of male cells (Moo-Valle et al. 2004; Macías-Macías and Quezada-Euán 2015).

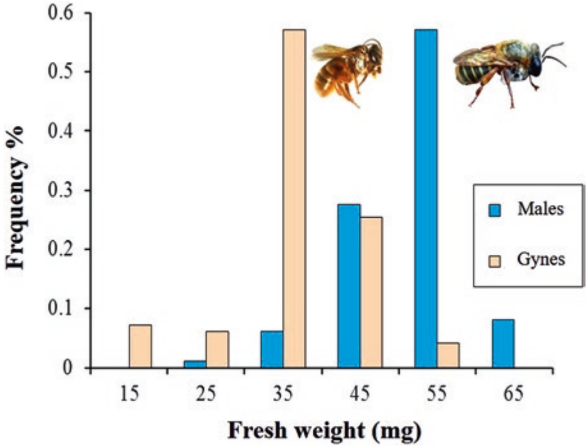
Male production in colonies of stingless bees is frequently associated with the amount of food reserves (Moo-Valle et al. 2001; Chinh et al. 2003). Colonies tend to produce more males when they have large food reserves. In *Melipona,* when food reserves dwindle, male production suffers more than queen production, and can drop to almost zero (Moo-Valle et al. 2001). Generally, males represent an additional cost because their production would be at the expense of more valuable workers (Velthuis et al. 2005). Nevertheless, it is interesting noting that a marked seasonality in the production of males is not evident in stingless bees, in contrast to *A. mellifera*. For instance, in *M. beecheii* it is possible to find males in colonies all year round (Moo-Valle et al. 2001). In stingless bees asynchrony in the production of males and gynes seems a common feature of colonies. However, asynchronous production of males and gynes in a population, will lead to the simultaneous occurrence of both sexes from different colonies when mating occurs (Moo-Valle et al. 2001; Chinh et al. 2003; de de Bruijn and Sommeijer 2006).

In highly eusocial bees, gene dispersal via males could be cheaper compared with colony fission, in which many workers depart with the queen in a swarm (Roubik 1990). Overall male production in stingless bees (as in other eusocial Hymenoptera) is on average larger than gyne production (the numerical sex ratio is male biased), even in species with excess production of gynes, like in *Melipona.* In *M. beecheii* for instance, annual male production is twice that of gyne production (Moo-Valle et al. 2001). In other stingless bees, male bias could be even larger because fewer queens are produced. For instance, in the case of *Sch. quadripunctata,* the production of males to gynes is in the range of 200:1 (Velthuis et al. 2005). At the moment of mating, the rate of males to gynes can increase even more, because many queens (particularly in *Melipona*) can be sacrificed before they mate. The production of males to gynes at the time of mating can be easily in the range of 1000:1 (Engels and Imperatriz-Fonseca 1990). Under these conditions, in *Melipona* the probability of mating for individual males is in the range of 0.01–0.002 (Velthuis et al. 2005).

Evidently, excessive Male-biased production in stingless bees represents stark competition for females. However, the males of highly eusocial bees do not compete among themselves using strength, displacement, or fighting (Engels and Imperatriz-Fonseca 1990). This means that selection by females seems scarce or nonexistent; rather, males seem to engage in a scramble competition for queens (Paxton 2005). Nonetheless, some male physical characteristics could be advantageous in scramble competition for reproduction. In *M. beecheii* for instance, male fresh weight can vary between 25 and 65 mg, representing a weight difference of nearly threefold among individuals in the same population (Bustillos 2016; Fig. 6.7).

Although the benefit of large size has not been shown in stingless bees, there is evidence from other Apidae, that larger males may have mating advantages (Schlüns et al. 2003; Couvillon et al. 2010). Likewise, it may be possible that larger, or heavier stingless bee males, can more readily chase or get hold of gynes.

Fig. 6.7 Distribution of male and gyne fresh weight (mg) at emergence in *M. beecheii* colonies from Yucatan, Mexico (Bustillos 2016)



On the other hand, small males may also have reproductive advantages. For instance, small males of *Scp.* aff. *depilis* persist for longer periods in male congregations. It is speculated that persistence may increase their opportunities for mating, but no correlation was found between body size and sperm viability in males persisting in a congregation (Koffler et al. 2016). Nonetheless, it seems that stingless bee males could be selected for endurance (Roubik 1990). It could also be possible that small males persist longer in a mating congregation because small size restricts dispersal over long distances; note, though, that it is not known if larger males tend to disperse over longer distances. Although males of different sizes can follow contrasting reproductive strategies in other bee species (Alcock 1997), it remains to be shown, if the striking differences in body size found in male stingless bees, may have different adaptive value.

The resources of a colony may impact male traits. Males *of M. beecheii* reared in colonies with large pollen reserves are larger and produce more spermatozoa, compared with males reared in colonies with lesser reserves (Pech-May et al. 2012; Fig. 6.8).

In stingless bees, the male reproductive tract is formed by the structures for the production and storage of sperm, plus the copulatory organ or genital capsule (Fig. 6.9). As in other bees, spermatogenesis in male stingless bees takes place during the pupal stage and, when individuals emerge from their cells, the process is completed (Dallacqua and Cruz-Landim 2003). This means that males emerge with a fixed number of spermatozoa and no additional production occurs in adulthood. However, males emerge sexually immature; the spermatozoa in their testes must migrate to the seminal vesicles before mating and insemination can effectively

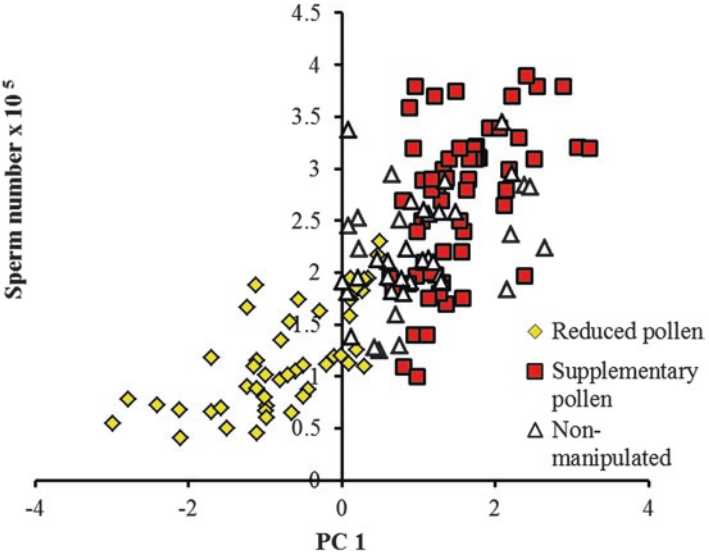


Fig. 6.8 Sperm production in three groups of *M. beecheii* males reared in colonies with reduced pollen reserves, excess pollen, and colonies non-manipulated (natural). PC1 is a multivariate measure of body size obtained from three morphometric traits (Pech-May et al. 2012)

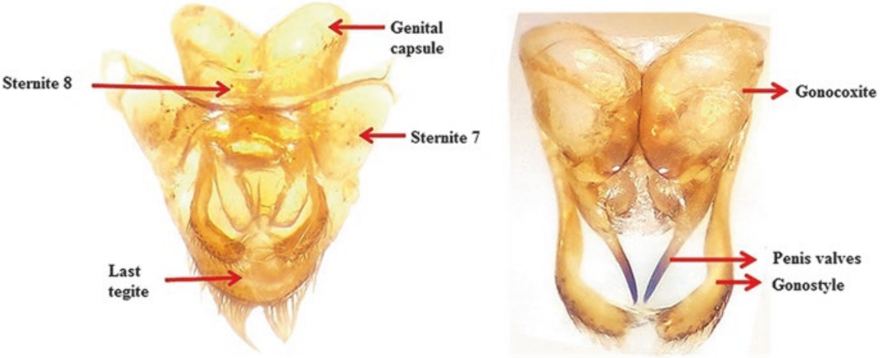


Fig. 6.9 The genitalia (left) and genital capsule (right), dissected from a male of *M. beecheii*

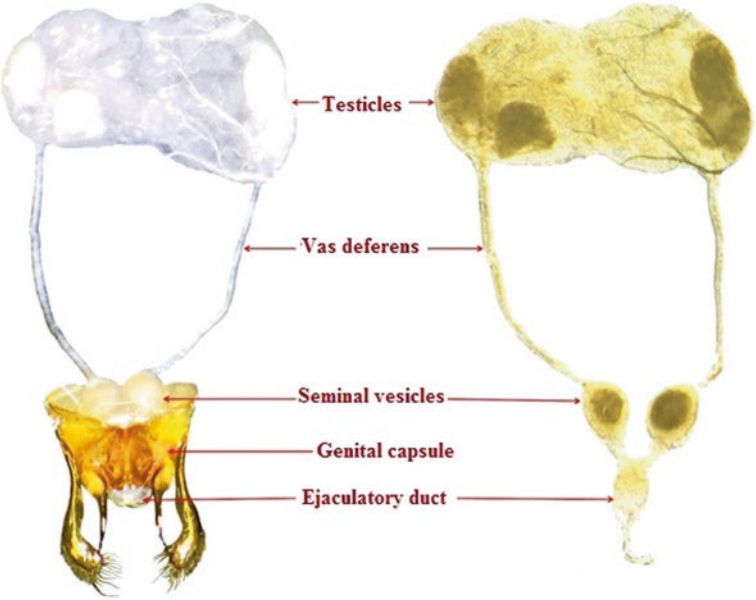


Fig. 6.10 Genital capsule and internal reproductive organs of a male of *M. beecheii*. The image on the left shows the testicles surrounded by the scrotal capsule. The image on the right shows the genital capsule removed and some migration of spermatozoa (dark spots) into the seminal vesicles

occur (Chapman 1998). Young males, recently emerged from their cell, have enlarged testes and the tubules (*vas deferens*) connecting the testicles with the seminal vesicles, are distended. As individuals age, the spermatozoa migrate to the seminal vesicles, and the testes gradually become atrophied in sexually mature individuals (Brito et al. 2010) (Figs. 6.10 and 6.11). The testes of stingless bees have four seminiferous tubules each (Ferreira et al. 2004) (Fig. 6.11).

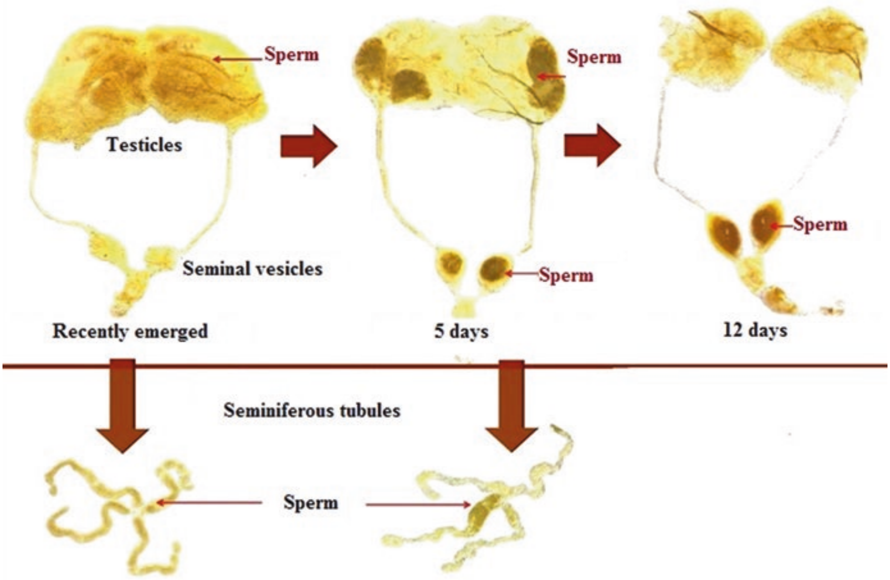


Fig. 6.11 The process of male sexual maturation male sexual maturity in *M. beecheii*. The image to the left shows a recently emerged male with no presence of spermatozoa (dark areas) in the seminal vesicles. The image in the center shows the start of the migration of the spermatozoa, which is almost finished in the male of the image to the right. In the image below, sperm concentrates in the confluence of the four seminiferous tubules of each testicle in five days old males, when migration starts to the seminal vesicles

In contrast with other Apidae, the male reproductive tract of stingless bees has simple genital ducts and lacks accessory glands (Ferreira et al. 2004; Colonello and Hertfelder 2005). In other bees, male accessory glands produce secretions that serve as nutrients for the spermatozoa in their movement along the female tract (den Boer et al. 2009). These secretions can also modify the post-copulatory behavior of the female (Dallacqua and Cruz-Landim 2003; Ferreira et al. 2004). Because of the lack of accessory glands in stingless bees, it is believed that substances helping the survival of the spermatozoa are found in other sections of the male reproductive tract (Dallacqua and Cruz-Landim 2003; Brito et al. 2010). In addition to anatomical simplification, the reproductive system of male stingless bees has comparatively thicker *vas deferens* and the ejaculatory duct is short. This type of elementary reproductive apparatus (compared with males of other taxa), lacking accessory glands is exclusive to the male Meliponini, and has been classified as type IV (Ferreira et al. 2004) (Fig. 6.11).

When stingless bee males emerge, they remain in their nest for a period of time before reaching sexual maturity, after which they abandon the nest and start an independent life, never returning to their natal colony (Roubik 1990). The time males spend in their colonies is variable, and is related with the speed at which they reach sexual maturity. In *M. beecheii* males can be sexually mature at 18 days, but there is considerable variation, ranging between 6 and 27 days (Camposeco 2005). Intra-colony factors could be related with the speed at which males reach sexual maturity. For instance, *M. beecheii* males that were reared in colonies with little pollen reserves, reached maturity later, compared with males that were reared in colonies with surplus pollen stores (Pech-May et al. 2012). However, in the same species, the presence of spermatozoa in the seminal vesicles was evident on the fifth day of age, in spite of the nutritional regime at which males were reared (Pech-May et al. 2012). In other *Melipona* species, like *M. compressipes*, males reach sexual maturity between 11 and 14 days of age (Kerr 1996), in *M. scutellaris* at 9–11 days (Abreu and Penna 1992), and in *M. quadrifasciata* at 8–12 days (Camargo 1972).

In males of *M. mondury* (Lima et al. 2006) and *Sc. xanthotricha* (Araújo et al. 2005), sperm can be seen in the vesicles 4 days after their emergence, but in the case of *M. quadrifasciata,* this occurs only by day 7 (Camargo 1984), and in *Frs. schrottkyi,* by day 9. The latter, is the longest period registered for the start of sperm migration in stingless bees (Brito et al. 2010). It is not known if surplus nutrition in adult males could accelerate sperm migration and sexual maturity.

The amount of sperm produced in male stingless bees is variable between, but also within species. In *M. quadrifasciata* individual sperm production is in the range of 1–1.2 × 10^6 (Kerr et al. 1962). In *M. beecheii* sperm production can vary among males between 1 and 3 × 10^6 (Pech-May et al. 2012), and in *Scp.* aff. *depilis* an average of 1.4 × 10^6 spermatozoa per male has been registered (Meneses et al. 2014).

Before reaching sexual maturity, males are mostly inactive in the nest (van Veen et al. 1997). Nevertheless, in contrast to honey bee males, they can engage is some activities (nectar dehydration, wax production, collection of pollen, and propolis), and can be indirectly involved in others, such as colony defense (Cortopassi-Laurino 1979; Boongird and Michener 2010; Schorkopf 2016). Nogueira-Neto (1997) considers that the activities performed by stingless bee males are related more to their preparation to leave the nest, rather than a real contribution to the colony. In this regard, it is males older than 20 days which normally engage in nectar dehydration (van Veen et al. 1997*;* van Veen 1999). In *M. beecheii*, van Veen (1999) observed that, as males age, they tend to spend more time in the area of food pots, which seems in agreement with the suggestion by Nogueira-Neto (1997).

The possibility of resin and pollen collection by males of some stingless bee species is surprising. It has been proposed that this behavior may occur more frequently in species in which males have hind tibiae comparatively similar to the workers'. In such species males are called isopodal, to identify them from species in which males and workers have very different hind legs, which are called anisopodal (Boongird and Michener 2010). Whether males of isopodal species are recruited to collect food, or if they do it independently, is still unknown.

When reaching sexual maturity, stingless bee males leave their natal colony (van Veen 1999), after which they survive by feeding on flowers (Kerr et al. 1962; Pers. Obs.). Eventually, males can join one aggregation, or perhaps move between several of these male groups (Fig. 6.12). In *Ttr. dorsalis,* males that were individually marked in an aggregation, remained in it for only a short time (Roubik 1990), which suggests that they may search for a particularly suitable one, or move constantly among different aggregations (dos Santos et al. 2016). Moving among aggregations could increase their opportunities for mating.

Fig. 6.12 Aggregation of *N. perilampoides* males. The behavior of the males at aggregations alternates between flying in a cloud that moves around colonies (left), and resting nearby (image to the right)

Two forms of male aggregations have been proposed in stingless bees. In species other than *Melipona*, males actively search for virgin females, and they congregate nearby colonies with young gynes (Fig. 6.12). In contrast, in *Melipona* it is believed that it is the gynes that search for male aggregations, which seem to be located far away from colonies (Velthuis et al. 2005). Nonetheless, *Melipona* male groups have also been reported close to meliponarios (Sommeijer et al. 2004; dos Santos et al. 2014), and individual males can be seen perching nearby colonies (Pers. Obs.).

In the case of non-*Melipona* species, when a gyne is accepted in a colony, males start to congregate nearby a few days after (Pers. Obs.). How males detect the presence of gynes in such colonies is still a matter of speculation. It has been suggested that males at long distance perceive some kind of queen pheromone, or that foragers carry queen smell, indirectly guiding males to their colony (Roubik 1990).

Male aggregations are formed by hundreds or thousands of males which originate from a large number of colonies, some located at considerable distances (dos Santos et al. 2016). Molecular analyses of males at aggregations have revealed the number of colonies from which they originate. In the case of *Scp. mexicana* males at aggregations originate from 21 to 55 colonies (Kraus et al. 2008; Mueller et al. 2012), up to 70 in *Ttr. angustula* (dos Santos et al. 2016), and up to 132 in the Asian *Ttga. collina* (Cameron et al. 2004). It is important to note that only a few males seem to originate from colonies nearby; as a rule the majority of males come from distant colonies (Mueller et al. 2012; dos Santos et al. 2016). Thus, male stingless bees seem capable of moving over long distances and, some studies have directly assessed this. Carvalho-Zilse and Kerr (2004) marked males of *M. scutellaris* and released them at different distances from their colony. Their results showed that some males can travel up to 1 km, although males released 100–400 m returned with more frequency to the meliponary. Using molecular markers, dos Santos et al. (2016) estimated the distance that *Ttr. angustula* males can disperse to be 600 m on average, but some dispersed over 2 km. It is important to note that such distances were calculated between two points in single events. It is possible that males travel considerably longer distances during their life span.

The behavior of males moving away from their natal colony and the fact that male aggregations are formed by males of multiple origin, seem effective adaptations to reduce the possibility of inbreeding. The wandering behavior of males is crucial in stingless bees, particularly considering that queens are predominantly monandrous, and that colonies are philopatric. The long dispersal of stingless bee males seems a key aspect to reduce inbreeding and effectively increase panmixia (Kraus et al. 2008). Recent findings of only subtle genetic differentiation among populations of some common species, like *Ttr. angustula* (Santiago et al. 2016) and *Pt. bilineata* (Landaverde-González et al. 2017), indicate the importance of male-mediated gene flow in stingless bees and the risk of losing genetic diversity if male dispersal is affected.

In the case of *Melipona,* male aggregations have been rarely seen. It has been suggested that males do not aggregate nearby colonies because although *Melipona* colonies constantly produce queens, most are killed. In this regard, it would not be adaptive for males to search for gynes near a colony (Sommeijer et al. 2004). Detailed reports of male aggregations have been provided on *M. favosa* and *M. subnitida* (Sommeijer and de Bruijn 1995; Sommeijer et al. 2004; dos Santos et al. 2014). The males of these species have been seen perching in groups, which contrast to the flying aggregations of males in *A. mellifera,* and non-*Melipona* stingless bees. Such perching sites have been found usually at 1.5 m height, where the number of males can be between 300 and 400. Similar to the aggregations of other stingless bees, the first males are seen early in the morning and the last just before dusk, but they do not overnight at the site (Sommeijer and de Bruijn 1995; Sommeijer et al. 2004). It is important to note that although the presence of queens has been reported in *Melipona* male aggregations, no attempts to mate have been observed (Sommeijer et al. 2004; dos Santos et al. 2014). It could be argued that these perching aggregations are resting males. In fact, observations in confinement chambers suggest that mating seems to take place in flight in *Melipona*, as in the other eusocial bees (pers. Obs.).

If stingless bee males and queens have some way to communicate their presence to each other is still under speculation (Engels 1987). It has been suggested that males of *M. favosa* release some kind of pheromone or chemical signal that attracts queens (Sommeijer et al. 2004). Interestingly, in *Scp. mexicana*, Galindo-López and Kraus (2009) showed that males were attracted to groups of other males. Curiously, in the field, males of that species were not attracted to colonies with virgin queens. The authors proposed that some type of chemical attraction among males could occur in this species. Interestingly, males of different species can attend the same aggregation. Interspecific similarities in the cuticular compounds and possible confusion caused by this type of recognition system have been dismissed as explanation for mixed aggregations of males (dos Santos et al. 2015). The explanation of mixed male aggregations is still unclear.

Interestingly, workers of *M. favosa* and *M. quadrifasciata* may also be found at male aggregations. It has been proposed that workers may attract males to these locations by means of pheromones or by bringing resin or other materials to these sites from their nests (Roubik 1990; Sommeijer et al. 2004; dos Santos et al. 2014).

As males are produced constantly in stingless bee colonies (Moo-Valle et al. 2001; de Bruijn and Sommeijer 2006), male aggregations can be active during most part of the year.

6.2 Colony Reproduction and Dynamics

The formation of new nests in many highly eusocial insects involves colony fission (reproductive division or swarming), although how this occurs varies across taxa (Table 6.1). In the case of stingless bees, colony fission occurs at low frequencies, and involves a long period of preparation. After swarming, a bond between mother and daughter nests can last months before they separate completely (Engels and Imperatriz-Fonseca 1990). In contrast, in honey bees, swarming occurs more frequently and rapidly, and does not involve further contact between mother and daughter colonies.

Swarming has rarely been observed in stingless bees; there are only few accounts on how it occurs (Engels and Imperatriz-Fonseca 1990). A prerequisite for swarming is large worker populations and food reserves. In colonies with these conditions, workers start searching for a potential new nest site nearby, sometimes in the same tree or substrate as the mother colony. This may explain the frequent aggregation of nests observed in many stingless bee species. In the honey bee, searching for a new site where the swarm will establish, only occurs after the bees have left the mother colony, and this can be located a kilometers away from the maternal nest (Seeley 1985).

Once a potential nest site has been selected, workers start conditioning the new cavity and build structures using cerumen and resin taken from the mother colony (Fig. 6.13). Within a few days, food pots are built and bees start moving pollen and honey from the mother colony too. Interestingly, Nogueira-Neto (1997) noted that the food carried by supplying bees is frequently a mixture of pollen and honey. Once stores have been developed in the new nest, swarming takes place. A virgin or a few virgin queens exit the mother colony with a group of workers that move into the new nest. Stingless bee swarming is different compared with honey bees, in which the mother queen leaves the nest before a virgin queen emerges (Winston 1987). Because the physogastric stingless bee queen cannot fly, it is the gynes that relocate with the swarms. At the new nest site, a young queen is selected and, after a few days, she performs her nuptial flight. The workers continue moving food and building materials from the mother colony until the new nest is self-sufficient (Sakagami 1982; Engels and Imperatriz-Fonseca 1990; Fig. 6.13).

The conservative system of swarming in stingless bees results in low rates of colony reproduction. The mother colony would need sufficient time (probably years) to accumulate the reserves needed to support her survival, and that of a daughter colony. The reproductive strategy of stingless bee colonies seems to be the production of few offspring (Slaa 2006) (Fig. 6.1). However, new stingless bee colonies seem to have high rates of survival. In a study in Costa Rica, the survival of

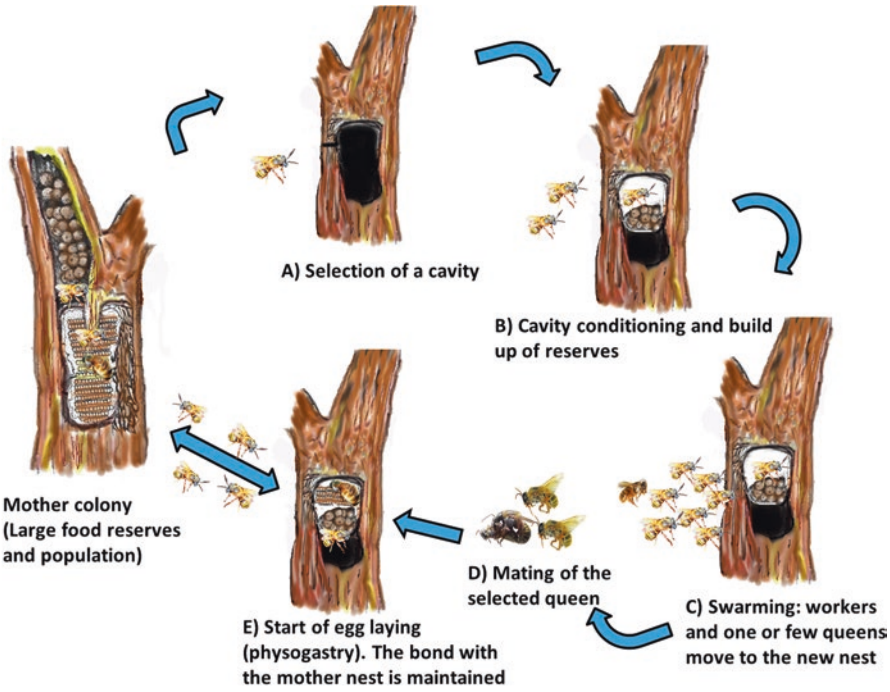


Fig. 6.13 The process of swarming (colony fission) in Meliponini. The letters and arrows indicate the progression of events

new stingless bee colonies was close to 70% at the end of the first year. In comparison, nearly all new Africanized honey bee colonies established in the same area died (Slaa 2006). In this regard, stingless bees seem to fit the description for K-strategists, characterized by monogamy, slow growth, and production of a few progenies, each with a high probability of survival. In comparison, r-strategists, like Africanized honey bees, have high fecundity and relatively little investment in individual progeny (Mac Arthur and Wilson 1967; Rinderer and Hellmich II 1991), but also experience high rates of mortality.

However, the strategy of colony foundation involving philopatry can be disadvantageous when habitats are altered. Fewer nesting sites or food resources can increase competition, but also the length of time necessary for colonies to reproduce (Engels and Imperatriz-Fonseca 1990; Brown and Albrecht 2001). The relatively limited dispersal of stingless bee colonies could also explain why populations of some species exhibit marked genetic structure for maternal rather than biparentally inherited genetic markers (Quezada-Euán et al. 2012: Santiago et al. 2016).

Meliponini colonies may take years for swarming (Roubik 1989), depending on the amount of reserves and building materials accumulated. In each annual cycle, colonies may gain reserves and increase size during the flowering season, but lose some during dearth, depending on the severity of shortage (Roubik 1982; Quezada-

Euán et al. 2015). Colonies may probably experience alternative episodes of increasing and losing size, until they reach a status capable of sustaining mother and daughter colonies (Quezada-Euán et al. 2015). Large stingless bee species, like *Melipona,* could be more sensitive to habitat alteration because they need comparatively more food and spacious cavities to reproduce (Brown and Albrecht 2001; Macías-Macías et al. 2014). Various species of *Melipona* have been considered under threat and risk of extinction (Kerr 2002); *M. beecheii* is one of them, especially in the Mayan region of southern Mexico (Quezada-Euán et al. 2001; Villanueva-Gutiérrez et al. 2013).

In the Yucatan, Santos (2006) surveyed the diversity of colonies in a deforested northern part of the Peninsula. No nests of *M. yucatanica* or *M. beecheii* were found. In contrast, small meliponine nests of *Fr. nigra* and *N. perilampoides* were common. This may be because these species are less demanding in the size of cavities or food, they need for successful nesting (Samejima et al. 2004).

Interestingly, the longevity of small stingless bee species in pristine forests can be shorter when compared to deforested regions, or urban areas (Slaa 2006). For instance, the annual survival of nests of *Ttr. angustula* in a forest was estimated at 0.74, but in deforested areas it increased to 0.92. Moreover, the life span of colonies of this species has been estimated to be 12.5 years in deforested areas but only 3.8 years in forests (Slaa 2006).

The estimated natural density of stingless bee colonies varies between a few to several nests per ha (Hubbell and Johnson 1977; Roubik 1983; Eltz et al. 2002; Antonini and Martins 2003; Roubik 2006). Such contrasting findings in the density of nests seem a result of two main factors, food resources and suitable spaces for nesting (Eltz et al. 2002). In Costa Rica, Hubbell and Johnson (1977) reported that food availability seemed to be the main factor limiting the density of colonies in forests. Similarly, a study in Southeast Asia detected that food (mainly pollen) was the factor with the highest impact on nest densities. However, the availability of nesting sites also had a significant effect, albeit to a lesser extent (Eltz et al. 2002). In contrast, in a different forest of Costa Rica, Brosi (2009) found a more marked link between suitable nesting sites and density of colonies. In Mexico, Macías-Macías and Quezada-Euán (2015) in a cloud tropical forest of western Jalisco, found that the availability of large cavities in trees (183 cm or greater in diameter) was positively related to the density of *M. colimana* colonies. Thus, nest and food availability are probably linked. In a given area, if food is scarce even with sufficient nesting sites, colony density would decline, and *vice versa*. Nonetheless, habitat conservation should increase food availability and the opportunities for nesting. Indeed, the highest diversity of colonies of different species has been found in preserved forests (Hubbell and Johnson 1977; Batista et al. 2001; Samejima et al. 2004). In addition to the negative effect of deforestation, human practices, like honey hunting, could represent an additional threat to the survival of stingless bee colonies (Batista et al. 2001; Kerr 2002; Slaa 2006).

It seems that the best strategy for the conservation of stingless bees is the preservation of species diversity by maintaining sufficient numbers of colonies. Diversity could be greatly enhanced by preserving patches of forest, even in highly deforested regions, that serve as corridors connecting distant areas, allowing the transit of reproductives and the survival of colonies (Brosi et al. 2007; Fischer et al. 2008; Landaverde-González et al. 2017).

6.3 Sexual Dimorphism

The concept of sexual size dimorphism (SSD) refers to the consistent difference in average body size of males and females within a species (Fairbairn 1997). SSD can be estimated using variables related to body mass (weight) and also linear measurements (dimension). In animals, SSD could be male or female biased, depending on which sex is larger. In species in which both sexes are of similar size SSD is therefore, close to zero (Fairbairn 1997).

It is assumed that body size differences can be subject to selection (natural or sexual), that differentially affect one sex over the other (Fairbairn 1997). If the pressure of selection upon fertility is strong, then females become larger than males. However, if sexual selection acting upon males is relatively more important, then SSD should be male biased (Teder 2005). In mammals and birds where males control a group of females, SSD is male biased. In contrast, in most insect species SSD is female biased, which reflects more intense selection for fertility on females, than sexual selection on males (Blanckenhorn et al. 2007).

Not much is known about the patterns of SSD in bees, and this is most evident in the highly social species. In a comparative study, Shreeves and Field (2008) evaluated the effect of parental care on SSD in solitary bees and wasps. The studied species were classified into two groups, those that build their own nest, and parasite species that do not build nests nor forage. The results showed that in both groups SSD was female biased, but that it was more pronounced in species that build nests, than in parasites (Shreeves and Field 2008). It seems that the selection pressure acting on female fertility is stronger in females that build nests. Parasite females released from the pressure of building a nest have experienced less selection for large body size. Interestingly, the study also revealed that female-biased SSD is less intense in bees compared with other Hymenoptera, possibly because of the comparatively minor load represented by pollen in relation with prey (Shreeves and Field 2008).

Recently, the pattern of SSD was evaluated in eusocial corbiculate bees (*A. mellifera, M. beecheii*, and *E. viridissima*) to test if, similar to solitary species, SSD is female biased (Medina et al. 2016). The results, using fresh weight and dimensions of virgin queens and males at emergence, showed that the highly eusocial species had a moderate male-biased SSD (Medina et al. 2016). In the case of the primitively eusocial *E. viridissima*, males and females were of similar dimensions, but males were slightly heavier. It is interesting that in both highly eusocial species (*A. mellifera, M. beecheii*), males were larger than females. One possible explanation for these results is that, similar to parasite solitary females, the queens of highly eusocial species have lost the pressure of nest building and provision, tasks that are now performed by the workers (Medina et al. 2016). Nonetheless, these results are preliminary, and more species should be studied to determine if this pattern is similar across stingless bees, and other highly eusocial species.

One proximate cause of SSD in corbiculate bees is the relative rate of development of males and females. In species where females develop faster (protogyny), females are usually smaller than males, resulting in male-biased SSD (Teder 2014). Both, the queens of *A. mellifera* and *M. beecheii* develop faster and emerge before males. In contrast, in *Bombus*, another member of the corbiculate bees, queens develop slower and are larger than males (Duchateau and Velthuis 1988; Cueva del Castillo and Fairbairn 2012). Interestingly, stingless bee gynes other than *Melipona* are larger than males and, it is suggested that they also take longer to develop (Wille 1983; Gonzalez-Acereto 2008).

Although preliminary, the evidence suggests two patterns of SSD in stingless bees (Fig. 6.14). On one side, *Melipona* gynes develop faster and are of smaller size and weight than males. On the other side, gynes of species different to *Melipona* are larger than the males (and workers), and seem to take longer to develop (Figs. 6.14 and 6.15). These differences result in male-biased SSD in *Melipona,* but female-

Fig. 6.14 Two patterns of sexual dimorphism in stingless bees: in *M. beecheii* the male on the left is larger than the gyne on the right. The reverse is found in *Scp. pectoralis*

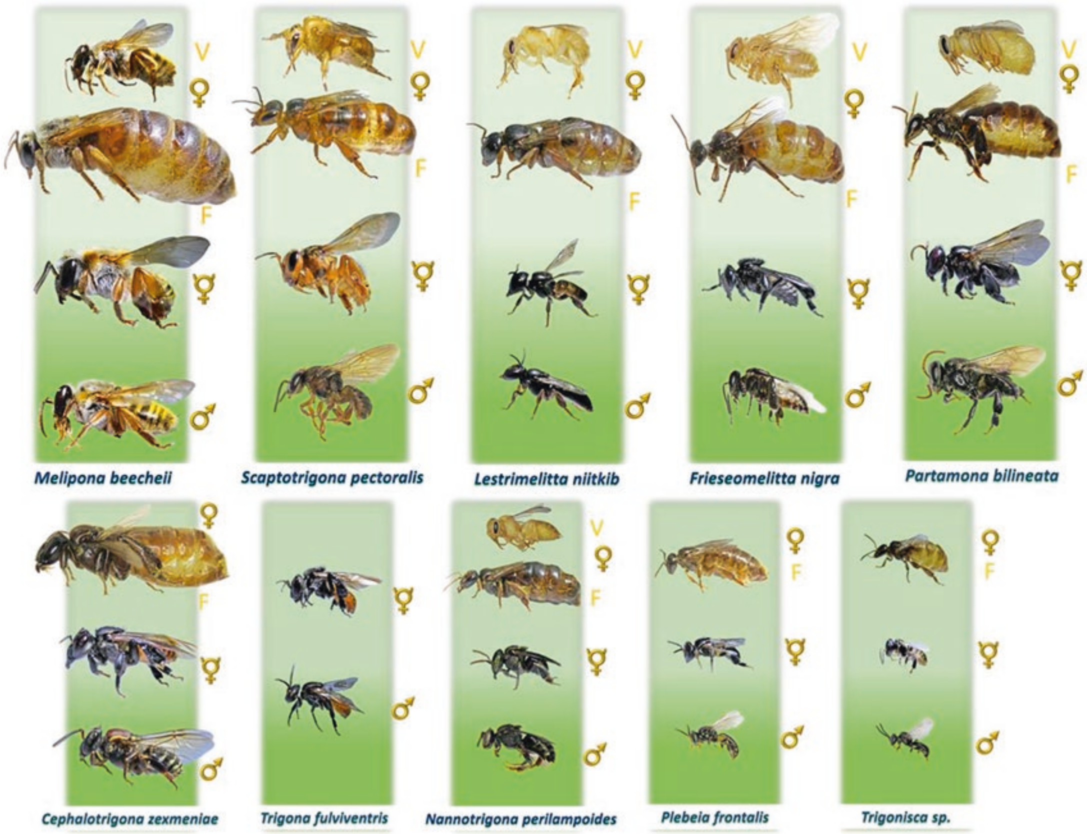


Fig. 6.15 Differences in body size among sexes and castes of various species of Meliponini from Yucatan. *V* virgin queen, *F* physogastric queen

biased SSD in other stingless bees (Fig. 6.15). Finding explanations to these contrasting patterns should be important to better understand the evolution of reproductive strategies in stingless bees.

6.4 Commercial Reproduction of Colonies

Stingless bee colonies normally reproduce slowly and they need a few months to be self-sufficient (Slaa 2006). However commercial use of colonies requires methods to facilitate a sustainable mass production over time (Quezada-Euán et al. 2001; Slaa 2006).

One way to rapidly increase colony numbers is through the division of more than one daughter from a single mother colony. For commercial purposes, an effective way would be using few workers and combs in what are caled mini-divisions (Imperatriz-Fonseca 1977). In Yucatan, studies with *M. beecheii,* have shown five main aspects to consider in the production of mini-colonies in this species (Quijano et al. 2008):

- The mother colonies used in the production of mini-divisions should have a young queen and large population and reserves.
- The mother colony should not have been harvested in the previous season, and should be fed with sugar syrup (and possibly some pollen supply), for at least 3 months to maintain large adult population and good rates of brood production.
- A worker number for a successful mini-division is ~200 young workers and a comb with ~100 pupae. It is recommended that young naive workers are used, because forager bees will return to the mother colony.
- Thermoregulation is crucial, so excess space in mini-hives should be avoided or reduced. Small boxes (10 × 15 × 7 cm) made of thick wood (of at least 3 cm) are recommended.
- Divisions should be well protected against the invasion of phorid flies. Mosquito nets may be used to keep them closed for 2 or 3 days, and use vinegar traps if necessary (see Chap. 8). Artificial feeding with pasteurized syrup is also recommended after 1–2 days (sugar in water 1:1 or 2:1).
- If possible provide cerumen for the construction of structures.
- Supplying food pots should be avoided for a few days until the division is organized.
- On a commercial scale, the use of temperature chambers could increase the success of the mini-divisions (Pers. Obs.). It would be important to further evaluate this alternative method of mass production of stingless bee colonies.

In the case of *Melipona* species, queen availability for the mini-divisions is not a limiting factor; normally, there are surplus queens. However, for species other than *Melipona,* the presence of a queen or a queen cell is essential for the success of mini-divisions. The workers of most non-*Melipona* bees cannot produce emergency

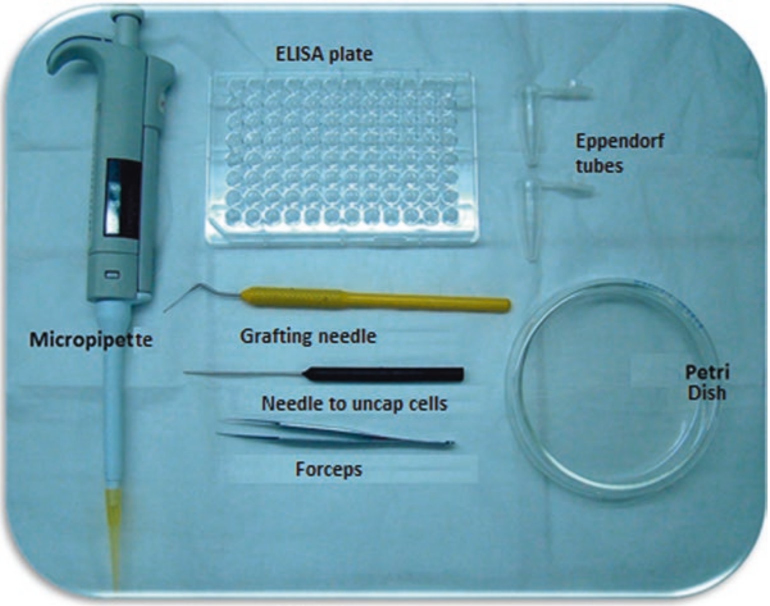


Fig. 6.16 Materials used for artificial queen rearing in non-*Melipona* stingless bees. The materials should be sterilized prior to use

queens (Sakagami 1982). Queen availability is therefore an important factor limiting the mass production of colonies in such species.

Recently, methods to artificially rear queens of non-*Melipona* stingless bees have been developed. Prato (2010) successfully raised queens after transferring young *Ttr. angustula* larvae to ELISA acrylic plate wells containing 55 μL of larval food (Fig. 6.16). Baptistella et al. (2012) evaluated the survival and size of queens in *Fr. varia* produced using 61–62 μL of larval food (2.3 times the amount needed to rear a worker). They found that grafting larvae older than 1 day produced small low quality queens (Menezes et al. 2013). In *Scp. depilis* artificial queen rearing was successful using 134 μL of larval food (Menezes et al. 2013).

It seems that one crucial factor for artificial queen rearing in stingless bees is humidity. The recommended humidity is 75% and temperature is 28° ± 0.5 °C (although the latter requires adjustment considering the ideal temperatures for each species). Another consideration is the care of larvae when transferring them from the cell to the acrylic plate. Larvae have soft bodies that can be easily damaged. In addition, they need to be placed on the food in the same position that they were when removed from their cells.

It is important remembering that, when larvae hatch, they lie on the larval food on one of their sides. The spiracles in contact with the food are obstructed and the larvae use the spiracles on the opposite side to breath. If larvae during transfer are

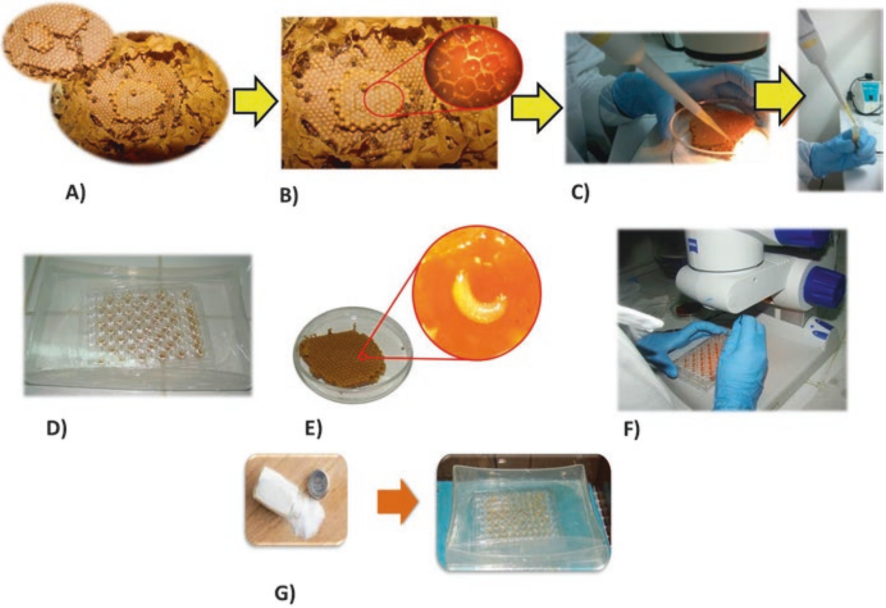


Fig. 6.17 Artificial production of queens of *Scaptotrigona pectoralis*. The letters refer to the different steps described in the text.

placed on the side that has not been in contact with the food, they will not be able to breath and will die (Baptistella et al. 2012; Menezes et al. 2013).

In Yucatan, we have used the basic methods of Baptistella et al. (2012) and Menezes et al. (2013), with some modifications (Figs. 6.17 and 6.18), to rear queens of *Scp. pectoralis*, *N. perilampoides*, and *L. niitkib*. The general process is described in the following sections (see Fig. 6.17); the amount of food and the temperature are the main factors that vary between species:

1. Two strong colonies must be used; one will serve as the donor of larval food and the other of young larvae. It is not recommended to obtain larvae and food from the same colony because it can reduce its strength.
2. All materials should be sterilized using hot water or UV light for 15 min. For the collection of larval food, one or two new combs (<2 days sealed) should be removed from the donor colony, and transferred to the lab or a room under clean conditions. The caps of the cells are removed using forceps, opening four to six cells at a time to avoid desiccation of the liquid food. The eggs on top of the food should be discharged.
3. The larval food is collected using a micropipette. The food is deposited in Eppendorf tubes (1.5 mL), and homogenized using a vortex apparatus (at ca. 3200 rpm). If the larval food is not used immediately it can be stored for a few days at −20 °C (Prato 2010).

4. Acrylic ELISA-type plates are recommended as artificial combs. The plate is usually 1.5 cm thick (8.4 × 7.6 cm) with a total of 96 cells, each with 4 mm diameter and 8 mm depth, similar to the cells of the three species mentioned above. The amount of larval food used to rear queens in *Scp. pectoralis* has been estimated at 86 μL (Gutiérrez et al. 2016), and for *L. niitkib* and *N. perilampoides* 63 μL and 42 μL, respectively (Pers. Obs.). The corresponding amount of food is deposited in alternate cells on the ELISA plate to avoid contamination of neighboring cells in case they are invaded by fungi. Once the plate has been supplied with larval food, it is placed in a room or an incubator at 28 °C with 100% relative humidity for 24 h. That level of humidity can be best achieved by placing the plate in a tray containing 50 mL of water, wrapping both with plastic film. This step is important to keep the larval food moistened and also for its stratification as occurs in nature, before larvae are grafted (Menezes et al. 2013).
5. On the day of grafting, a new comb (<24-h-old larvae) is extracted from the donor colony. It is important to have registered the exact age of larvae because, as mentioned before, larvae older than 1 day would result in less perfect queens.
6. In this case, comb cells are opened, one at a time. With a grafting needle similar to that used in honey bee queen rearing, each larva is carefully removed from its cell, and deposited in one of the ELISA plate cells supplied with larval food. The grafting needle should have a smooth surface to avoid damaging the larva. After all the larvae had been grafted, the plate is kept for additional 4 days at 28 + 2 °C and 100% relative humidity, which is achieved as explained in step D. The larval food should be moistened to allow adequate consumption by the larvae. If the food desiccates, larvae will not be capable of consuming the total amount and workers or imperfect queens are produced (pers. Obs.).
7. If larval food has been totally consumed after 4 days, the humidity is decreased to ca. 75%, by adding NaCl (18 g of common salt to 50 mL of water in the tray). If the food has not been consumed, one more day could be given to the larvae at 100% humidity. However, monitoring the humidity is important. Under excess humidity, fungi could proliferate, but if humidity is insufficient, the larval tegument may dry (loss of the creamy, shiny aspect), in which case adding more water to the tray is recommended. Larvae should be monitored every day.

When individuals enter the pupal stage, the plates can be checked every 3 days (Fig. 6.18). After approximately 44 days, gynes of *Scp. pectoralis* complete their development. Artificially produced gynes can be placed in small plastic boxes, with a group of workers and syrup in Eppendorf tubes, where they can live up to 2 weeks (pers. Obs.). Significant advances have been made to understand the reproductive strategies of stingless bees. Manipulating reproduction opens the possibility of preserving species and populations, and also for commercial use. However, significant gaps are still left to fully comprehend reproduction in these insects that may support sustainable management.

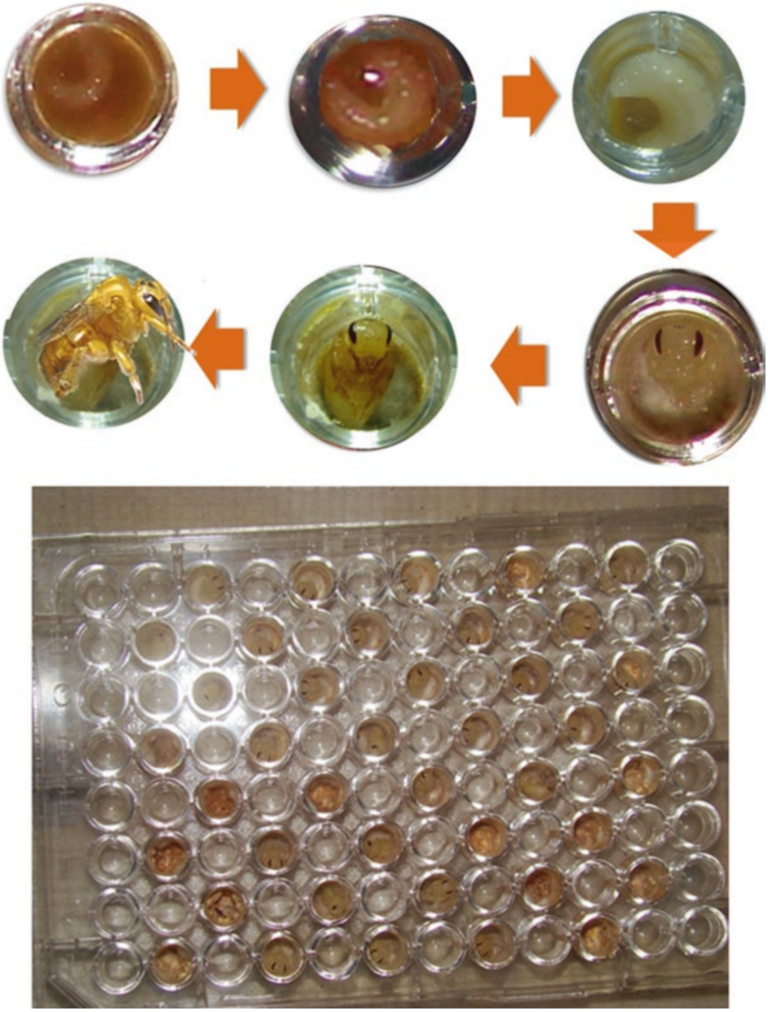


Fig. 6.18 Above: Different phases of artificial *S. pectoralis* gyne production. Below: ELISA plate with *S. pectoralis* gynes in their final pupal stage

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