Flexible morphology and task allocation in workers of the stingless bee *Frieseomellita paupera* (Hymenoptera, Meliponinae)

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Division of labour according to age (age polyethism) has been thoroughly studied in the honeybee *Apis mellifera*. We investigated the division of labour and the relationship between age, task allocation and pigmentation in the stingless bee *Frieseomellita paupera* Prov. (Apidae, Meliponinae). In this species newly hatched workers are completely pale, whereas fully developed individuals are completely black. We compared task allocation and pigmentation in established colonies with a laying queen and a steady production of young bees, with those in a colony that lacked an influx of young bees. In conditions with an interrupted production of young bees, older workers retained tasks normally performed by younger nest mates. Strikingly, these older workers additionally showed a colouration of the abdomen, usually exclusively seen in younger workers. This can be explained by the typical biology of this species and by hypothesizing a system of task allocation in which workers patrol the colony to find tasks that need to be done. We assume that only bees that are not fully coloured have an abdomen that is sufficiently elastic to allow for storing a large amount of larval food which is essential for performing brood rearing activities.

Keywords: age related behaviour, foraging for work, pigmentation, task allocation, *Frieseomellita paupera*

One of the main characteristics of eusocial insects is a distinct division of tasks between the members of the colony. In the social Hymenoptera, the separation between the female members is most conspicuous: the queen (or queens) has the single task of producing eggs and the workers perform all other duties necessary for nursing the offspring and maintaining the colony. In eusocial bees, workers build brood cells and other nest structures, forage for food and other commodities, process the incoming food and prepare it for use by nest mates or for storage, rear sibling worker and male larvae, feed the queen, defend the nest against enemies, and clean the nest.

In several species of stingless bees (Meliponinae) the division of labour among workers was found to be largely comparable to that in *A. mellifera*, the young workers predominantly performing hive duties as cleaning and nursing the brood, and older workers being the foragers [*Plebeia* (*Plebeia*) *droyana* (Terada *et al.* 1975), *Scaptotrigona postica* (Sakagami *et al.* 1982), *Melipona favosa* (Sommeijer 1984)]. Recently a morphological separation was found between guard bees and foragers in *Tetragonisca angustula* (Grüter *et al.* 2012).

The most prominent difference in task allocation between stingless bees and *A. mellifera* can be found in behaviours concerning brood rearing. Sommeijer (1984), marked *M. favosa* workers individually, and found that the tasks, of cell building and provisioning of brood cells, which in *Apis* are performed by different age classes, are in this stingless bee not only performed by the same age class, but are interconnected and performed on the same day by the same individuals. This difference between *Apis* en *M. favosa* is attributed to the different systems for building brood cells and rearing brood.

Both in *Apis* and Meliponinae, the age dependent system of allocating tasks is to some extent flexible. Worker bees react by changing their normal behaviour when the colony is stressed and another effort of the individuals is needed. In a colony of *M. favosa* with a small influx of young bees, we found that individuals that participated previously in brood cell building and provisioning can revert to these tasks at an older age. That the relation between age and tasks may vary according to colony conditions is also noted by others (Bego & Simões 1972, Sakagami *et al.* 1982, Kolmes & Sommeijer 1992). The flexibility not only extends to behaviour, but also implies physiological adaptations (Simões, 1974).

In most stingless bee species the recently emerged workers have a different colour than older workers and are therefore called callows. Sakagami (1982) describes a general pigmentation scenario for stingless bees: nurse bees, being older than a few days, are intermediately pigmented and foragers (older still) are completely pigmented (*cf.* Bassindale 1955).

The process of pigmentation probably is connected to the process by which the chitin skin hardens in the course of time. In the genus *Melipona*, this process is rather quick (a few days) and inconspicuous, with only minor colour differences. In the tribe Trigonini the pigmentation process is more conspicuous. Here, callows usually are of a very light colouration, sometimes almost white, whereas fully developed foragers can be pitch-black. In these species the pigmentation process takes a longer period of time. Bassindale (1955) discriminated between five pigmentation stages in *Trigona gribodoi* Magretti, each worker went through these stages over time. For *Trigona* (*Tetragonula*) *laeviceps*, Salmah *et al.* (1983) described a strict relation between age and pigmentation, that made it possible to determine workers age by classifying her pigmentation stage.

In the slender neotropical stingless bee *Frieseomellita paupera* Prov. [former name: *Trigona* (*Tetragona*) *nigra paupera*], we also found a relation between the body pigmentation and the tasks that are performed by workers. As in *T. gribodoi* Magretti, nurse bees of *F. paupera* that engage in brood cells building and provisioning, are exclusively half way in the pigmentation process: possessing pale abdomens and black heads and thoraces. In this species foragers are always entirely black (Sommeijer *et al.* 1984).

Frieseomellita paupera is within the stingless bees further characterised by a series of typical features concerning brood care and colony multiplication. Colonies of this species have a tendency to swarm more readily than many other species and nests can be found in all kinds of nesting places (Fig. 1).

Brood production is concentrated in a single 'integrated oviposition process' (IOP, *cf.* Sakagami 1983), which typically takes places once per 24 h, in the dark hours of the day. In this IOP, up to about 35 cells are being provisioned and oviposited by the queen. At the start of the IOP all newly constructed cells are being filled synchronously with larval food. Provisioning of a single brood cell in this species consists of a relatively small number of food discharges (mean ± SD = 3.5 ± 0.6; Sommeijer *et al.* 1984). The entire process is performed in just a few minutes. Unlike many other species of stingless bees, workers of *F. paupera* do not oviposit during IOP, nor do they at any other time in their lives.

The objective of our study is to analyse a number of the phenomena that set these bees apart from other groups of stingless bees. By integrating these phenomena, we attempt to improve the understanding of their functional significance. In this paper we present the result of a study on the ontogeny of the colouration process in *F. paupera*. The extent of the flexibility of this process was

Figure 1. Unstable *Frieseomellita paupera* nesting sites. Left: A nest in a cardboard beer bottle case; right: Nest remains in the inside of a plywood door.

studied by placing colonies of this species in a stress situation. The role of the flexibility of this process, usually thought of as relatively autonomous and rigid, in the typical biology of *F. paupera* will be discussed.

MATERIAL AND METHODS

Colony origin and maintenance

Frieseomellita paupera is a common species on the islands of Trinidad and Tobago and is also reported from Venezuela, Colombia, Panama and Costa Rica (Moure *et al.* 2007). We performed our studies on Tobago, located near the coast of Venezuela (11°15'N, 60°40'W). The island has a tropical climate including a dry and a rainy period and with an average daily temperature of around 32 °C. Observations were carried out just after the rainy season, from the end of August to March, in stable ambient conditions.

Colonies were collected from cavities (for instance in trunks of tropical almond trees or in brick-walls) and were subsequently transferred to wooden boxes (measuring 30 × 14 cm).The boxes were placed inside a building, in order to be able to protect them from weather influences and predators. After a period of habituation the bees were allowed to forage outside, through a plastic tube running through the wall of the building. To be able to observe bees without disturbing them, each box was covered with glass and observations were done using red light.

Observed colonies

For this study we used three colonies: two regular colonies (col #1 and col #2) and one temporarily de-queened colony (col #3). The two regular colonies each contained a laying queen and workers of all age classes. In these colonies there was a stable influx of young bees. In the temporarily de-queened colony we removed the queen for a period of 24 days. By doing so, we disrupted the production and subsequent emergence of young bees. After this period the queen was reintroduced. On the day of reintroduction of the queen, still a number of young bees emerged that were produced in the period before the de-queening, but their numbers decreased rapidly, resulting in a shortage of superseding young workers.

Age marking

To be able to assess the relationship between age, behaviour and colouration, we marked all newly hatched workers of one particular day. By connecting the hive to an annex in which mature brood was placed to hatch, we were able to easily catch and mark all bees that emerged in 1 day. Workers were provided with small dots of paint on their thorax. Thus, in each of the colonies col #1 and col #2 a cohort of 34 young bees were marked, and in col #3 of 20 bees. All of these workers were completely pale in colouration.

Observation methods

In all colonies we made an inventory of the colour patterns, running from the white new-borns (callows) up to the entirely black workers. The colour of head, thorax, and abdomen, as well as of the legs, was assessed both dorsally and ventrally.

Workers of different colouration as well as age-marked workers were traced inside the hive during the day time in three daily observation periods of 40 min each. Of all observed bees the activity was noted down to be able to assess the participation of workers of certain age and/or colouration class in tasks, related to brood rearing, maintenance of the colony structures and foraging. The observations of workers of different colouration classes were done in a period of 2 months, the individually marked workers were traced for as long as they were seen present in the colony.

In the evenings, similar observations were done, but the duration of the observations was longer at this time of day, to be able to also observe workers' involvement in cell construction, 'royal court' formation and IOP behaviour.

RESULTS

Stages of pigmentation

By scanning for colouration patterns in a colony of *F. paupera*, we were able to obtain an inventory of possible colouration patterns. We placed them in order of increasing pigment (Fig. 2). This scheme will be referred to as the 'pigmentation-scenario'.

Tasks performed by colouration classes

Strikingly, the division of activities over the various pigmentation stages was similar for all observed colonies, in both of the two regular colonies #1 and #2 as well as in the manipulated colony #3 (Table 1). WW workers participate in

Table 1. Performance of behaviour activities by workers of different colouration.

Stage	Activities
WW	Patrolling, trophallaxis, pollen processing
GW-I	Cell nibbling, cell construction, pot nibbling, pollen processing
GW-II	Cell nibbling, cell construction, pot nibbling, pillar construction, wax processing, pollen processing
GW-III	Cell nibbling, cell construction, cell discharge, pot nibbling, pillar construction, wax processing, collecting of debris and its transfer to foraging bees, pollen processing
BW	Same as for GW-III
BG	Pot nibbling, pillar construction, wax processing, fly out, ventilating
BB	Same as for BG

trophallaxis and pollen nibbling, BW workers are particularly important in the construction and provisioning of brood cells and BB workers are foragers.

Age related task participation

Figure 3 shows the age related participation in tasks performed in the regular colonies col #1 and col #2. Brood rearing activities (brood cell building, discharging of larval food) and Royal Court behaviour are performed at an age of 4-15 days with a peak between 9 and 11 days. Building and maintaining nest structures other than brood cells is performed between 3 and 44 days, and foraging starts at an average age of 23 days.

The colouration process in both regular colonies and in colonies with an interruption of influx of young bees

The graphs in Figure 4 show the relation between age and pigmentation stage for age marked workers. In the regular colonies #1 and #2, marked workers were seen up to an age of 44 days. After 6 or 7 days all of the observed worker bees (N = 22 and 21 in col #1 and #2, respectively) were in the GW-stage. In 3 weeks time no more GW workers were found, and 36% and 29% of the observed marked workers (N = 28 and 21, respectively) were of the BW colouration and about 20% reached full pigmentation. After 6 weeks all observed bees were BB (N =28 and 24 for col #1 and #2, respectively).

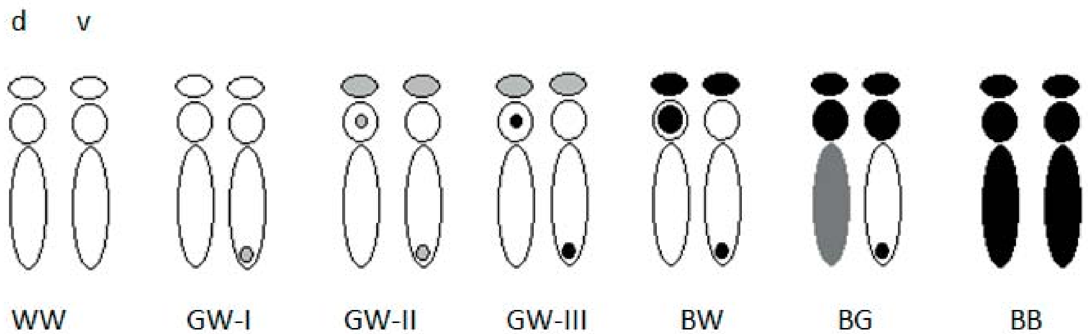

Figure 2. Pigmentation-scenario based on the distinct pigmentation-patterns for worker-bees in *Frieseomellita paupera* colonies (d, dorsal side; v, ventral side). WW: Just emerged worker bees, completely pale head, thorax and abdomen. The legs are half black/white. GW-I: Pale head and thorax, pale abdomen with brown tip on ventral caudal side. The legs are half black/white. GW-II: A brown head, a small pale brown plate on thorax (dorsal side) and a pale abdomen. The legs are half black/white. A small brown tip at the caudal end, ventral side of abdomen. GW-III: A grey head, a small black plate on thorax (dorsal side) and a pale abdomen. The legs are half black/white. A small black tip at the caudal end, ventral side of abdomen. BW: A black head, a big black plate on thorax (dorsal side) and a pale abdomen. The legs are half black/grey. A small black tip at the caudal end, ventral side of abdomen. BG: A black head, a completely black pigmented thorax. The abdomen is pale on the ventral side and grey/black on dorsal side. The legs are fully black pigmented. BB: A fully pigmented *Frieseomellita* worker-bee (completely black).

In contrast, in the queen deprived colony (col #3) there is a striking delay in the colouration process. In this colony the half way colouration stage BW was seen to prevail up to 44 days in the final week in which marked bees were seen. In all observations done 2, 4, 6 and 7 weeks, more than half of the observed

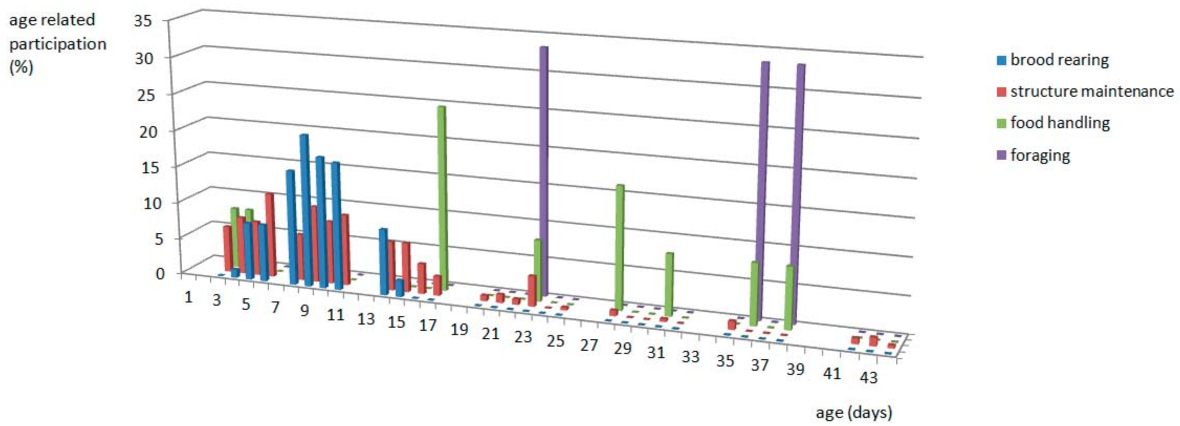

Figure 3. Age dependant participation in various tasks performed in hives with a regular influx of young workers.

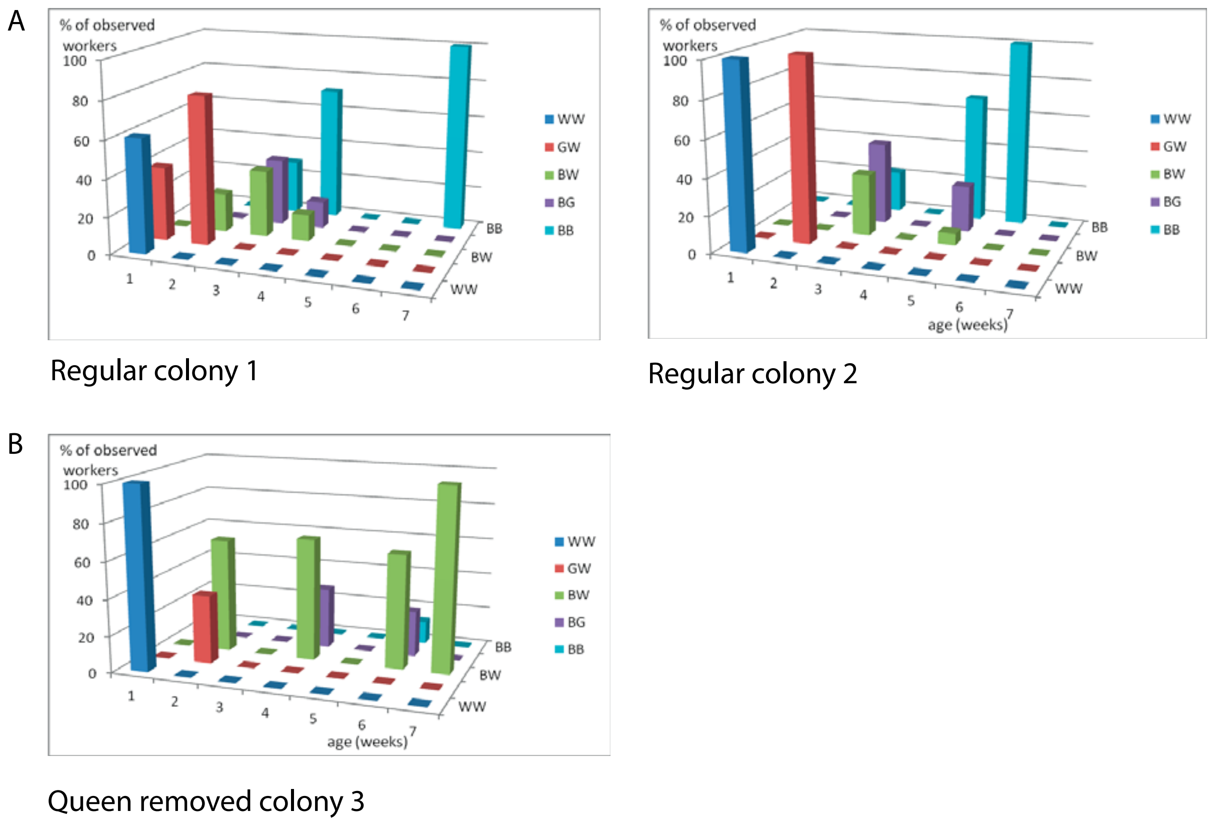

Figure 4. Age dependant colouration of workers in two (regular) colonies with a steady influx of young bees (A) and in a colony with a disrupted influx of young bees (B). In both regular colonies there is a succesion of colouration stages running from WW through BW to BB in 6-7 weeks. In the colony with a disrupted influx of young bees after 3 weeks the BW stage prevail up to an age of 7 weeks.

marked bees still were in BW stage. At 44 days only four marked workers were left and they all were half coloured (BW). These bees died without becoming fully pigmented.

Thus, the first steps in the pigmentation process appeared to be similar for colonies #1, #2 and #3. During the first 17 days all bees, regardless of colony conditions, went through the same pigmentation stages from completely pale to a stage in which the head and thorax are black, and the abdomen pale (half coloured stage). Subsequently, in the regular colonies (#1 and #2) with a stable influx of young bees most workers became fully pigmented after 3 weeks. However, in colony #3, the half coloured stage was prolonged up to an age of 44 days in all observed bees but one.

DISCUSSION

Flexibility in both behaviour and physiology

Our results show that in *F. paupera* both worker behaviour and the ontogeny of the pigmentation of the cuticle are not firmly depending on age, but instead are flexible. In established thriving colonies, with a stable influx of young bees, there is a clear correlation between worker age and activities performed. At 9 days of age there is a peak in brood activities. At 3 weeks of age the first bees start foraging. In our experimental colonies there was a prolonged performance of brood activities by workers of a far older age class, up to an age of 57 days.

These results are in line with the concept of 'foraging for work' (Franks *et al.* 1994): workers patrol the colony and find tasks that need to be done. Recently, another example of physiological flexibility related to task allocation was found in A. *mellifera* (Baker *et al.* 2012). In this species, the authors established that learning ability can recover in aged foragers that revert to nursing tasks. They found that this recovery is positively associated with levels of stress response/cellular maintenance proteins in the central brain.

The process of pigmentation appeared to probably consist of two sub phases:

1. An autonomous phase. In the first 17 days of their lives, all workers pass through similar colouration stages and reach the half coloured phase, possessing black heads and thoraces and white abdomens. This development is independent of colony conditions, we found it in all normal colonies we observed and also in both experimental colonies, which indicates a genetically fixed first half of the process.

2. A flexible phase. This may last from 4 days up to more than 40 days, according to colony needs. At the end of this phase workers may sometimes reach full pigmentation, but some half coloured workers, that maintain this colouration pattern for the longest time, may never get fully colourized. We have found these workers to die as a half coloured bee. As half coloured bees of very high age were never found in all regular colonies we observed, we can rule out the possibility of the colour variation being an expression of genetic polymorphism.

The physiological regulation of these phenomena is yet unclear. Several insect hormones are known to affect the development of the cuticle and its coloration as well as insect behaviour. A likely candidate for regulating both the coloration of the cuticle as well as worker behaviour in *F. paupera* is Juvenile Hormone (JH). This hormone is known to affect the pace at which a honey-bee worker (*A. mellifera*) develops into a forager (Sullivan *et al.* 2000). In this species JH titers progressively increase through the first 15 or so days of the worker's life before the onset of foraging (Elekonich *et al.* 2001). Furthermore in other insect species, JH regulates the synthesis of phenoloxidase (Hiruma & Riddiford 1984), which in bees is important in the production of melanine, and through this in the process of cuticle pigmentation (Zufelato *et al.* 2004).

The function of flexible behaviour and pigmentation

Frieseomelitta paupera is a very successful species, both on the island of Trinidad and on Tobago. Nests are very common and are found in a many nesting sites. The flexibility we found in the task performance of workers is functional for the high swarming frequency of this species. A recently established swarm implies a disruption of emergence of workers since the new queen still has to start laying. With a daily production of some tens of brood cells and a normal worker-participation in 'discharging larval food' of a few days, every day a number of about 90-120 workers must be available for this task. Smaller or larger disruptions in the influx of bees demands for a task allocation, not firmly exclusively (strictly) depending on age. In addition to this behavioural flexibility also a flexible abdomen is needed. *Frieseomelitta paupera* is a species with only 3.5 larval food dischargers per brood cell, each of these individuals discharging only once. Furthermore, trophallaxis is rare in this species, and surrounding workers do not 'refill' discharging workers during IOP. This is very different from, *e.g.*, *M. favosa* (Sommeijer *et al.* 1985). In *M. favosa* many trophallactic actions take place during POP's, enabling individual dischargers to discharge several times during a single POP. Therefore in *F. paupera*, each discharging individual must be able to contain an 'exceptionally' large amount of larval food at the start of the IOP. Light colored abdomens of *F. paupera* nurse-workers are larger than the black abdomens of foragers. An additional observation showed that bees with white abdomens were able to ingest much more liquid food than those with black abdomens.

We suggest that a regular shortage of young workers to supersede their elderly sisters (*e.g.*, as a consequence of natural swarming) is a strong selective force for the evolution of a flexible task allocation system, to secure more continuous brood production.

The absence of laying workers, another typical aspect of the biology of *F. paupera*, appears also to be related to the low number of discharges of larval food in brood cells. Large amounts of larval food kept in the extended honey stomach, leave little room for fully activated ovaries in a worker. The intriguing diversity in reproductive behaviour of stingless bees must be subject for evolutionary studies. Comparing the occurrence and regulation of worker oviposition combined with worker participation in larval feeding between major stingless bee groups, may be necessary for understanding social behaviour in bees.

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