

Reproduction in honeybee colonies

Symposium organized by Stephen Martin and Peter Neumann

Egg removal behaviour in honeybees (*Apis mellifera* L.)

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In honeybee, *Apis mellifera*, colonies with a queen worker reproduction is usually very low. However, workers are in principle able to lay unfertilized eggs which usually develop into haploid males. Kin selection theory provided a complex but plausible explanation for the lack of worker reproduction based on relatedness arguments. In light of worker policing theory queen and worker-laid eggs are differentially removed because workers are more related with the queens' than other workers' offspring. However, recent data show that queen and worker-laid eggs have differential survival rates suggesting that other reasons, e.g. the viability of the eggs, play an important role.

We tested the egg viability and egg removal behaviour of three honeybee subspecies: *A. m. carnica* and *A. m. scutellata*, where worker policing should occur and one (*A. m. capensis*) in which theoretically worker policing should be absent because laying workers produce diploid clonal female offspring. It has been shown that worker policing occurs in all three subspecies and that there is substantial worker reproduction possible in the African subspecies. In all subspecies queen-laid eggs were removed significantly faster than worker-laid eggs and queen-laid eggs were significantly more viable than worker-laid eggs. The different viability of queen and worker-laid eggs is the most parsimonious explanation for the differential removal of queen and worker-laid eggs due to classic hygienic behaviour.

Socially parasitic Cape honeybee workers suppress the development of their offspring

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Socially parasitic Cape honeybee workers, *Apis mellifera capensis*, can reproduce despite the presence of a host queen. Here we investigate the pheromonal and ovarian development of Cape honeybee workers in queenright *A. m. scutellata* host colonies in the course of infestation. Host colonies ($n=8$) were artificially infested with freshly emerged (0-24 h) parasitic workers ($n=157 \pm 11/\text{colony}$, 1st parasitic introduction). Four of the eight colonies were successfully infested. Brood frames containing the first parasitic generation were placed in incubators. Freshly emerged marked parasitic workers were reintroduced into their respective host colonies ($n=100/\text{colony}$, 2nd parasitic introduction). Samples of the 1st ($n=73$) and 2nd ($n=32$) introduced parasites were taken 1, 2, 3, 4, 5 and 6 weeks after introduction and the pheromonal and ovarian development was assessed. The data show that the parasites introduced initially had more activated ovaries and produced significantly more queen-like mandibular gland pheromones than those introduced second ($p<0.001$). It seems that initially introduced parasitic workers can pheromonally suppress the development of subsequent parasitic generations. Thus, our results explain earlier findings that only few parasitic reproductive workers are found in infested *A. m. scutellata* colonies.

Rare royal families in honeybees

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In honeybee colonies (*Apis mellifera*) the queen is the dominant female, controls reproduction and suppresses the ovarian development of workers. Queen larvae are selected by the worker bees and are fed a special diet, the royal jelly, which determines caste. Since queens mate with an extraordinary large number of males honeybee colonies are constituted of many subfamilies, derived from the different patrilineages. As a consequence there is a huge conflict potential among the subfamilies over queen rearing. Here we show that queens of the Cape honeybee, *A. m. capensis*, are not reared at random but are preferentially reared from rare 'royal' subfamilies, which have extremely low frequencies in the colony's worker force but a high frequency in the queens reared.

Absence of nepotism in the worker-queen interactions during secondary swarming in the honey bee

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In the honey bee, *Apis mellifera*, high polyandry leads to the presence of many subfamilies or patriline. When the colonies reproduce through swarming, workers rear a number of new queens, only a few of which will ultimately head a colony. Workers can then potentially increase their inclusive fitness if they can favour full-sister over half-sister queens during the queen rearing and elimination process. Most studies have so far focused on interactions between workers and queens who have emerged from their queen cells or during the rearing process. However, when afterswarms are produced, adult queens often remain in their queen cells for up to one week. In this situation, workers prevent the queens from emerging and protect them from other emerged queens. These beneficial behaviours could be a perfect stage for nepotism. This study makes the first ever investigation on the kinship between workers and adult queens who have not emerged from their queen cells. We observed the full suite of behaviours expected during this phase of colony reproduction, including piping from the queens. Although there was no evidence for nepotism in the worker-queens interactions, there was a non-random distribution across patriline of the workers attending the queen cells. In addition, in one colony we found differential treatment of fostered (non-kin) queen cells.

Fatal combat of queens in the genus *Apis*: Verification of a fighting pheromone and its interspecific efficacy

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In *Apis mellifera*, supernumerary queens are produced in the course of colony reproduction. Conflict among these sister queens is known to occur after swarming when they compete for reproductive dominance. As a rule, young *A. mellifera* queens fight after emergence and only one queen survives the aggressive encounters assuring the monogyny. We examined whether the aggressive behaviour between virgin queens is common in other *Apis* species and if queen's releasers of aggressive behaviour are effective interspecifically. Further we determined which stimuli elicit this aggressive behaviour, the location of the stimuli and at which age they occur. Finally we isolated and chemically characterised the releaser pheromone involved. Behaviour of virgin queens towards various species, ages, or treated queens and dummies was observed in a

bioassay and evaluated using stinging behaviour as a qualitative response. Queens of *A. mellifera*, *A. cerana*, *A. koschevnikovi* and *A. florea* showed similar behaviour within and between the species. We found that the stimuli responsible for releasing aggressive behaviour depended on contact with the dorsal surface of the abdomen. Moreover we demonstrated that tergites placed on a glass body are sufficient to release stinging behaviour. Extractions of the biological activity succeeded with nonpolar solvent. Using preparative GC we could verify the biological activity in one small fraction of the extract. Comparison of this queen fraction with the fraction of a worker using GC/MS and MALDI resulted in a single compound. Identification and synthesis of the new pheromone is in progress.

Survival of Asian honeybee spermatozoa in European honeybee queen

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European honeybee queens (*Apis mellifera*) were inseminated with spermatozoa of Asian honeybee drones (*A. cerana*, *A. florea*, and *A. dorsata*) and of *A. mellifera* (as control). Three days and four weeks after insemination, the motility (spermatozoa survival was observed by its motility) of spermatozoa in spermatheca of *A. mellifera* was monitored. There was no significant difference in spermatozoa motility in spermatheca within the two hive nesting species (*A. mellifera* and *A. cerana*) and within the two free nesting species (*A. dorsata* and *A. florea*). There was also no significant decrease in motility of *A. mellifera* and *A. cerana* spermatozoa in spermatheca within 4 weeks. The spermatozoa motility of *A. florea* and *A. dorsata* decreased after 3 days. Further decrease was observed after 4 weeks. Only drone offspring emerged from *A. mellifera* queens inseminated with heterospecific spermatozoa. The data show that only conspecific sperm survives which may be related to a species specific composition of the spermathecal fluid.

Genetic and behavioural analysis of nepotism in emergency queen rearing

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In the polyandrous honey bee, *Apis mellifera*, workers can potentially increase their inclusive fitness by rearing full-sister queens. If the mother queen dies suddenly,

workers feed a few larvae in worker cells with royal jelly and rear them into queens (emergency queen rearing). Using DNA microsatellite markers we determined the patriline of emergency queens reared in two colonies headed by naturally-mated queens before being made queenless. We also studied the direct behavioural interactions between marked workers and queen larvae whose kinship was determined afterwards. Although there were differences between the proportions of queens reared from each patriline in one colony, we found no influence of kin in the rearing process.

Relationship between larvae age and characteristics of queen honey bees (*Apis mellifera* L.) after single and double grafting

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The characteristics of queens were measured as an indicator and ninety mating colonies were used for this research. Single and double grafting applied for A1 and A2 grafting groups, respectively and 1, 2 and 3 days old larvae were grafted in each group. The average pre-oviposition periods were 12.41 ± 0.02 and 13.25 ± 0.04 days; the average queen weights at emergence were 181.8 ± 0.43 and 172.7 ± 0.61 mg; the average diameters of spermatheca were 0.97 ± 0.0001 and 0.98 ± 0.0010 mm and average numbers of spermatozoa were 4.500 ± 0.07 and 4.462 ± 0.09 for the queen bees of A1 and A2 groups, respectively. Queen weight at emergency, pre-oviposition period, diameter of spermatheca and the number of spermatozoa were not different within grafting methods. Larvae age had significantly ($p < 0.01$) effect on pre-oviposition period, diameter of spermatheca, the number of spermatozoa, and ($p < 0.05$) queen weight at emergency. A positive important ($p < 0.05$) correlation was observed between weights at emergency and the number of spermatozoa ($r = 0.508$). Additionally an important ($p < 0.05$) positive correlation ($r = 0.365$) was found between diameter of spermatheca and the number of spermatozoa.

An integrated model for honeybee population dynamics and hive management

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A model (ApiPop) to simulate the hive population dynamics and hive management is described. The integrated model (fig. 1) includes modules for honeybee population, hive management, enemies and poisoning, food availability and environmental effects. The model ApiPop was created by the SEMoLa language and

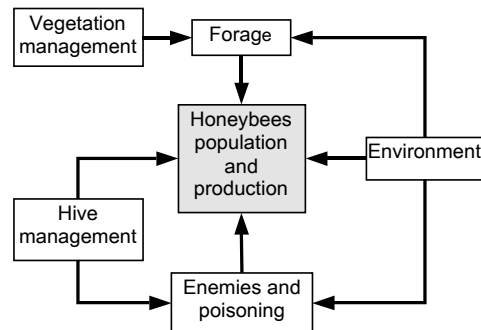
environment simulation (Danuso, 1999).

In the honeybee population model, the developmental cycle considers the following phases: before capping, after capping, adult phase (from emergence to death). Adult bees are divided into male (drones) and female (workers bees). In turn, worker bees are divided into house bees and foragers. Five causes of mortality are considered: natural, diseases and enemies (e.g., *Varroa destructor*), pesticides and environmental pollution, low temperatures and food shortage.

Regarding the foraging activity, the model is sensitive to the distance of the forage from the hive. The energy used collecting nectar, pollen and water, the quality of the food, and other factors influencing nectar production are also considered.

In the model, the rate of nectar harvest (g/hive/day) depends on the number of foragers, the average number of flights per forager per day and the amount of nectar gathered in each flight. The model ApiPop also takes into account the ageing processes, comb building, and the accumulation of nectar in the cells. The hive management practices (represented as events) considered in ApiPop are: insertion of empty supers, removal of full supers, the adding of more supers, new queen introduction, feeding with sugar and proteic feeds, colony combining, insertion of combs, comb foundations and diafragms.

The model can be used for didactical purposes and to improve hive management.



Reproduction in Cape honey bees, *Apis mellifera capensis*: Establishment of Cape honey bees as social parasites in African honeybee colonies

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Since Cape honey bees cause huge losses of African bee colonies by taking over reproduction, they are expected to establish easily after infecting a colony. However, by artificial introduction of marked bees, we found that African bees are by no means defenceless. Cape bees started to reproduce in only one of four colonies; in two other colonies all Cape bees were removed within three days. In the last African colony some of the Cape bees were removed, and the remaining Cape bees had not developed into egg-layers after 12 days. Apparently African bees can readily detect and remove potential Cape parasites after invasion into their colonies. Conditions inside the colony may be critical in defining vigilance of the bees. This suggests that alternative beekeeping management systems can reduce damage due to Cape parasites.