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The Regulation of Pheromones leading to Task Specialization in *Apis mellifera*

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ABSTRACT: The pheromones of the honey bee are mixtures of chemical substances released by individual bees into the hive or environment that cause changes in the physiology and behaviour of other bees. Honey bees (*Apis mellifera*) have one of the most complex pheromonal communication systems found in nature, possessing 15 known glands that produce an array of compounds.^{[1][2]} These chemical messengers secreted by a queen, drone, worker bee or laying worker bee to elicit a response in other bees. The chemical messages are received by the bee's antenna and other body parts. They are produced as a volatile or non-volatile liquid and transmitted by direct contact as a liquid or vapor.

Honey bee pheromones can be grouped into releaser pheromones which temporarily affect the recipient's behavior, and primer pheromones which have a long-term effect on the physiology of the recipient. Releaser pheromones trigger an almost immediate behavioral response from the receiving bee. Under certain conditions a pheromone can act as both a releaser and primer pheromone.

The pheromones may either be single chemicals or a complex mixture of numerous chemicals in different percentages.^{[3][4]}

KEYWORDS: *Apis mellifera*, pheromonal communication system, queen, drone, worker bee, chemical messengers, releaser and primer

I. INTRODUCTION

Types of honey bee pheromones

Two main alarm pheromones have been identified in honeybee workers. One is released by the Koschevnikov gland, near the sting shaft, and consists of more than 40 chemical compounds, including isopentyl acetate (IPA), butyl acetate, 1-hexanol, n-butanol, 1-octanol, hexyl acetate, octyl acetate, n-pentyl acetate and 2-nonanol. These chemical compounds have low molecular weights, are highly volatile, and appear to be the least specific of all pheromones. Alarm pheromones are released when a bee stings another animal, and attract other bees to the location and causes the other bees to behave defensively, i.e. sting or charge. The alarm pheromone emitted when a bee stings another animal smells like bananas.^[5] Smoke can mask the bees' alarm pheromone.¹

A queen bee is typically an adult, mated female (gyne) that lives in a colony or hive of honey bees. With fully developed reproductive organs, the queen is usually the mother of most, if not all, of the bees in the beehive.^[1] Queens are developed from larvae selected by² worker bees and specially fed in order to become sexually mature. There is normally only one adult, mated queen in a hive, in which case the bees will usually follow and fiercely protect her.³

The term "queen bee" can be more generally applied to any dominant reproductive female in a colony of a eusocial bee species other than honey bees. However, as in the Brazilian stingless bee *Schwarziana quadripunctata*, a single nest may have multiple queens or even dwarf queens, ready to replace a dominant queen in case of a sudden death.^[2]

During the warm parts of the year, female "worker" bees leave the hive every day to collect nectar and pollen.⁴ While male bees serve no architectural or pollinating purpose, their primary function (if they are healthy enough) is to mate with a queen bee. If they are successful, they fall to the ground and die after copulation.⁵ Any fertilized egg has the potential to become a queen. Diet in the larval stage determines whether the bee will develop into a queen or a worker. Queens are fed only royal jelly, a protein-rich secretion from glands on the heads of young workers. Worker larvae are fed bee bread which is a mixture of nectar and pollen. All bee larvae are fed some royal jelly for the first few days after hatching but only queen larvae are fed the jelly exclusively.⁶ As a result of the difference in diet, the queen will develop into a sexually mature female, unlike the worker bees.^[3]

Queens are raised in specially constructed queen cells. The fully constructed queen cells have a peanut-like shape and texture. Queen cells start out as queen cups, which are larger than the cells of normal brood comb and are oriented



vertically instead of horizontally. Worker bees will only further build up the queen cup once the queen has laid an egg in a queen cup. In general, the old queen starts laying eggs into queen cups when conditions are right⁷ for swarming or supersedure. Swarm cells hang from the bottom of a frame while supersedure queens or emergency queens are generally raised in cells built out from the face of a frame.

As the young queen larva pupates with her head down, the workers cap the queen cell with beeswax. When ready to emerge, the virgin queen will chew a circular cut around the cap of her cell. Often the cap swings open when most of the cut is made, so as to appear like a hinged lid.⁸

During swarming season, the old queen is likely to leave with the prime swarm before the first virgin queen emerges from a queen cell.⁹

Virgin queen bee

| Metamorphosis of the queen bee | |
|--------------------------------|---|
| Egg | hatches on day 3 |
| Larva (several moltings) | day 3 to day 8+ ¹ / ₂ |
| Queen cell capped | c. day 7+ ¹ / ₂ |
| Pupa | c. day 8 until emergence |
| Emergence | c. day 15+ ¹ / ₂ – day 17 |
| Nuptial flight(s) | c. day 20 – 24 |
| Egg laying | c. day 23 and up |

A virgin queen is a queen bee that has not mated with a drone. Virgins are intermediate in size between workers and mated, laying queens, and are much more active than the latter. They are hard to spot while inspecting a frame, because they run across the comb, climbing over worker bees if necessary, and may even take flight if sufficiently disturbed. Virgin queens can often be found clinging to the walls or corners of a hive during inspections.¹⁰

Virgin queens appear to have little queen pheromone and often do not appear to be recognized as queens by the workers. A virgin queen in her first few hours after emergence can be placed into the entrance of any queenless hive or nuc and acceptance is usually very good, whereas a mated queen is usually recognized as a stranger and runs a high risk of being killed by the older workers.¹¹

When a young virgin queen emerges from a queen cell, she will generally seek out virgin queen rivals and attempt to kill them. Virgin queens will quickly find and kill (by stinging) any other emerged virgin queen (or be dispatched themselves), as well as any unemerged queens. Queen cells that are opened on the side indicate that a virgin queen was likely killed by a rival virgin queen. When a colony remains in swarm mode after the prime swarm has left, the workers may prevent virgins from fighting and one or several virgins may go with after-swarms. Other virgins may stay behind with the remnant of the hive. Some virgins have been seen to escape the hive to avoid being killed and seek out another without a queen, such as in the eusocial bee *Melipona scutellaris*.^[4] This can contain multiple virgin queens.^[5] When the after-swarm settles into a new home, the virgins will then resume normal behavior and fight to the death until only one remains. If the prime swarm has a virgin queen and an old queen, the old queen will usually be allowed to live. The old queen continues laying. Within a couple of weeks she will die a natural death and the former virgin, now mated, will take her place.¹²

Unlike the worker bees, the queen's stinger is not barbed and she is able to sting repeatedly without dying.

Piping

Piping is a noise made by virgin and mated queen bees during certain times of the virgin queens' development. Fully developed virgin queens communicate through vibratory signals: "quacking" from virgin queens in their queen cells and "tooting" from queens free in the colony, collectively known as piping. A virgin queen may frequently pipe before she emerges from her cell and for a brief time afterwards. Mated queens may briefly pipe after being released in a hive.

Piping is most common when there is more than one queen in a hive. It is postulated that the piping is a form of battle cry announcing to competing queens and show the workers their willingness to fight. It may also be a signal to the worker bees which queen is the most worthwhile to support.

The adult queen pipes for a two-second pulse followed by a series of quarter-second toots.^[6] The queens of African bees produce more vigorous and frequent bouts of piping.^[7]

Reproduction cycle

The surviving virgin queen will fly out on a sunny, warm day to a "drone congregation area" where she will mate with 12–15 drones. If the weather holds, she may return to the drone congregation area for several days until she is fully mated. Mating occurs in flight. The young queen stores up to 6 million sperm from multiple drones in her spermatheca. She will selectively release sperm for the remaining 2–7 years of her life.^[8]

The young virgin queen has a limited time to mate. If she is unable to fly for several days because of bad weather and remains unmated, she will become a "drone layer." Drone-laying queens usually signal the death of the colony, because the workers have no fertilized (female) larvae from which to raise worker bees or a replacement queen.^[9]

Though timing can vary, matings usually take place between the sixth and tenth day after the queen emerges. Egg laying usually begins 2 to 3 days after the queen returns to the beehive, but can start earlier than this.^[10]

A special, rare case of reproduction is thelytoky: the reproduction of female workers or queens by laying worker bees by parthenogenesis. Thelytoky occurs in the Cape bee, *Apis mellifera capensis*, and has been found in other strains at very low frequency.^[11]

As the queen ages, her pheromone output diminishes. A queen bee that becomes old, or is diseased or failing, is replaced by the workers in a procedure known as "supersedure".^[13]

Supersedure may be forced by a beekeeper, for example by clipping off one of the queen's middle or posterior legs. This makes her unable to properly place her eggs at the bottom of the brood cell; the workers detect this and then rear replacement queens. When a new queen becomes available, the workers kill the reigning queen by "balling" her, clustering tightly around her. Death through balling is accomplished by surrounding the queen and raising her body temperature, causing her to overheat and die. Balling is often a problem for beekeepers attempting to introduce a replacement queen.^[14]

If a queen suddenly dies, the workers will attempt to create an "emergency queen" by selecting several brood cells where a larva has just emerged which are then flooded with royal jelly. The worker bees then build larger queen cells over the normal-sized worker cells which protrude vertically from the face of the brood comb. Emergency queens are usually smaller and less prolific than normal queens.^[15]

The primary function of a queen bee is to serve as the reproducer. A well-mated and well-fed queen of quality stock can lay about 1,500 eggs per day during the spring build-up—more than her own body weight in eggs every day. She is continuously surrounded by worker bees who meet her every need, giving her food and disposing of her waste. The attendant workers also collect and then distribute queen mandibular pheromone, a pheromone that inhibits the workers from starting queen cells.^[12]

The queen bee is able to control the sex of the eggs she lays. The queen lays a fertilized (female) or unfertilized (male) egg according to the width of the cell. Drones are raised in cells that are significantly larger than the cells used for workers. The queen fertilizes the egg by selectively releasing sperm from her spermatheca as the egg passes through her oviduct.^[17]



Identification

| Color | | Year ends in |
|--------|--|--------------|
| white | | 1 or 6 |
| yellow | | 2 or 7 |
| red | | 3 or 8 |
| green | | 4 or 9 |
| blue | | 5 or 0 |

The queen bee's abdomen is longer than the worker bees surrounding her and also longer than a male bee's. Even so, in a hive of 60,000 to 80,000 honey bees, it is often difficult for beekeepers to find the queen with any speed; for this reason, many queens in non-feral colonies are marked with a light daub of paint on their thorax.^[13] The paint usually does not harm the queen and makes her easier to find when necessary.¹⁸

Although the color is sometimes randomly chosen, professional queen breeders use a color that identifies the year a queen hatched, which helps them to decide whether their queens are too old to maintain a strong hive and need to be replaced. The mnemonic taught to assist beekeepers in remembering the colour order is Will You Raise Good Bees (white, yellow, red, green, blue).^{[13][14]}

Sometimes tiny convex disks marked with identification numbers (Opalithplättchen) are used when a beekeeper has many queens born in the same year - a method that can also be used to keep multiple bees in the same hive under observation for research purposes.^[15]

Queen rearing

Queen rearing is the process by which beekeepers raise queen bees from young fertilized worker bee larvae. The most commonly used method is known as the Doolittle method.^[16] In the Doolittle method, the beekeeper grafts larvae, which are 24 hours or less of age, into a bar of queen cell cups. The queen cell cups are placed inside of a cell-building colony.^[17] A cell-building colony is a strong, well-fed, queenless colony that feeds the larva royal jelly and develops the larvae into queen bees.^[18]

After approximately 10 days, the queen cells are transferred from the cell building colony to small mating nuclei colonies, which are placed inside of mating yards. The queens emerge from their cells inside of the mating nuclei. After approximately 7–10 days, the virgin queens take their mating flights, mate with 10–20 drone bees, and return to their mating nuclei as mated queen bees.^[17]

Queen rearing can be practiced on a small scale by hobbyist or sideline beekeepers raising a small amount of queens for their own use, or can be practiced on a larger, commercial scale by companies that produce queen bees for sale to the public. As of 2017, the cost of a queen honeybee ranges from \$25 to \$32.¹⁸

Beekeepers can also utilize alternative methods of queen rearing. Examples are the Jenter kit, walk-away split, Cloake board, and artificial insemination.



II.DISCUSSION

The other alarm pheromone is released by the mandibular glands and consists of 2-heptanone, which is also a highly volatile substance. This compound has a repellent effect and it was proposed that it is used to deter potential enemies and robber bees. The amounts of 2-heptanone increase with the age of bees and becomes higher in the case of foragers. It was therefore suggested that 2-heptanone is used by foragers to scent-mark recently visited and depleted foraging locations, which indeed are avoided by foraging bees. However, this has recently been proven false. In a new discovery, it was determined that bees actually use 2-heptanone as an anesthetic and to paralyze intruders. After the intruders are paralyzed, the bees remove them from the hive.^[6]

Another pheromone is responsible for preventing worker bees from bearing offspring in a colony that still has developing young. Both larvae and pupae emit a "brood recognition" pheromone. This inhibits ovarian development in worker bees and helps nurse bees distinguish worker larvae from drone larvae and pupae. This pheromone is a ten-component blend of fatty-acid esters, which also modulates adult caste ratios and foraging ontogeny dependent on its concentration. The components of brood pheromone have been shown to vary with the age of the developing bee. An artificial brood pheromone was invented by Yves Le Conte, Leam Sreng, Jérôme Trouiller, and Serge Henri Poitou and patented in 1996.^[7]

The Dufour's gland (named after the French naturalist Léon Jean Marie Dufour) opens into the dorsal vaginal wall. Dufour's gland and its secretion have been somewhat of a mystery. The gland secretes its alkaline products into the vaginal cavity, and it has been assumed to be deposited on the eggs as they are laid. Indeed, Dufour's secretions allow worker bees to distinguish between eggs laid by the queen, which are attractive, and those laid by workers. The complex of as many as 24 chemicals differs between workers in "queenright" colonies and workers of queenless colonies. In the latter, the workers' Dufour secretions are similar to those of a healthy queen. The secretions of workers in queenright colonies are long-chain alkanes with odd numbers of carbon atoms, but those of egg-laying queens and egg-laying workers of queenless colonies also include long chain esters.^[9]

Drone pheromone

Drone Mandibular Pheromone attracts other flying drones to suitable sites for mating with virgin queens.^[8]

Egg marking pheromone¹⁰

This pheromone, similar to that described above, helps nurse bees distinguish between eggs laid by the queen bee and eggs laid by a laying worker.

Footprint pheromone

This pheromone is left by bees when they walk and is useful in enhancing Nasonov pheromones in searching for nectar.

In the queen, it is an oily secretion of the queen's tarsal glands that is deposited on the comb as she walks across it. This inhibits queen cell construction (thereby inhibiting swarming), and its production diminishes as the queen ages.¹²

Forager pheromone

Ethyl oleate is released by older forager bees to slow the maturing of nurse bees.^[10] This primer pheromone acts as a distributed regulator to keep the ratio of nurse bees to forager bees in the balance that is most beneficial to the hive.

Nasonov pheromone

Nasonov pheromone is emitted by the worker bees and used for orientation and recruitment. Nasonov pheromone includes a number of terpenoids including geraniol, nerolic acid, citral and geranic acid.¹³

Other pheromones

Other pheromones produced by most honey bees include rectal gland pheromone, tarsal pheromone, wax gland and comb pheromone, and tergite gland pheromone.

Queen mandibular pheromone (QMP), emitted by the queen, is one of the most important sets of pheromones in the bee hive. It affects social behavior, maintenance of the hive, swarming, mating behavior, and inhibition of ovary development in worker bees.^[11] The effects can be short and/or long term. Some of the chemicals found in QMP are carboxylic acids and aromatic compounds. The following compounds have been shown to be important in retinue attraction of workers to their queen and other effects.^[12]



- (E)-9-Oxodec-2-enoic acid (9-ODA) – inhibits queen rearing as well as ovarian development in worker bees; strong sexual attractant for drones when on a nuptial flight; critical to worker recognition of the presence of a queen in the hive
- (R,E)-(-)-9-Hydroxy-2-enoic acid (9-HDA) promotes stability of a swarm, or a "calming" influence
- (S,E)-(+)-9-HDA¹⁴
- Methylparaben (HOB)
- 4-Hydroxy-3-methoxy phenylethanol (HVA)

Work on synthetic pheromones was done by Keith N. Slessor, Lori-ann Kaminski, Gaylord G. S. King, John H. Borden, and Mark L. Winston; their work was patented in 1991. Synthetic queen mandibular pheromone (QMP) is a mixture of five components: 9-ODA, (-)-9-HDA, (+)-9-HDA, HOB and HVA in a ratio of 118:50:22:10:1.

Queen retinue pheromone

The following compounds have also been identified,^[13] of which only coniferyl alcohol is found in the mandibular glands. The combination of the 5 QMP compounds and the 4 compounds below is called the queen retinue pheromone (QRP). These nine compounds are important for the retinue attraction of worker bees around their queen.¹⁵

- Methyl oleate^[14]
- Coniferyl alcohol^[15]
- Cetyl alcohol^[16]
- α -Linolenic acid^[17]

III.RESULTS

The queen also contains an abundance of various methyl and ethyl fatty acid esters,^[18] very similar to the brood recognition pheromone described above. They are likely to have pheromonal functions like those found for the brood recognition pheromone.

A laying worker bee is a worker bee that lays unfertilized eggs, usually in the absence of a queen bee. Only drones develop from the eggs of laying worker bees (with some exceptions, see thelytoky). A beehive cannot survive with only a laying worker bee.^[1]

Prevalence

Even in a normal hive, about 1% of workers have ovaries developed enough to lay eggs. However the usual number of the laid eggs is very small. Only eight eggs (seven moderately and one fully developed) were found after examining of 10,634 worker bees^[2] (strong colony contains about 100,000). Workers eventually lay significant numbers of eggs only in queenless colonies.¹⁶

Development

Laying workers develop in the absence of open brood as produced by a healthy adult queen. Normally, pheromones from the brood – known as brood recognition pheromones – prevent development of the workers' ovaries. Laying workers can develop after the colony's queen has been lost to swarming,^[3] or in the presence of a failing queen which has yet to be superseded. The process of developing a laying worker usually takes weeks after the loss of the original queen. In adult laying workers there is an anatomic (and physiological) trade-off between the sizes of their more developed ovaries and their less developed food glands.^{[4][5]}

IV.CONCLUSIONS

All methods of identifying a laying worker bee involve inspection, in which the beekeeper examines the brood pattern and type to identify if a healthy queen is present, or a potential laying worker. The beekeeper looks for diagnostic signs, including:¹

- Brood pattern Laying workers lay eggs that lack the queen's egg recognition pheromone, meaning that other workers may remove the eggs. This results in a spotty brood pattern, in which empty cells are scattered heavily through capped brood.



- Number of eggs per cell The beekeeper looks at the honeycomb cells to see how many eggs are laid in each one. Queen bees will usually lay only a single egg to a cell, but laying workers will lay multiple eggs per cell. Multiple eggs per cell are not an absolute sign of a laying worker because when a newly mated queen begins laying, she may lay more than one egg per cell.
- Egg position¹⁷ Egg position in the cell is a good indicator of a laying worker. A queen bee's abdomen is noticeably longer than a worker, allowing a queen to lay an egg at the bottom of the cell. A queen bee will usually lay an egg centered in the cell. Workers cannot reach the bottom of normal depth cells, and will lay eggs on the sides of the cell or off center.
- Drone brood in worker cells Another good indicator is drone brood in worker sized cells. Drones are raised in larger cells than workers. Drone cells are recognizable by their larger size; and when capped, drone cells are capped with blunt pointed cappings. Drones in worker cells are a sure sign of a failing queen or laying worker.¹⁸

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14. ^ methyl (Z)-octadec-9-enoate
15. ^ (E)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-ol ^ hexadecan-1-ol ^ (Z9,Z12,Z15)-octadeca-9,12,15-trienoic acid
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