Understanding Queen PHEROMONES

New discoveries explain some things, and ask more questions.

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New Information On The Queen Retinue Pheromone

What are Pheromones?

To understand queen bees – indeed to understand many beekeeping manipulations – you should have a good working understanding of pheromones produced by bees. Pheromones (Greek *pherein* = to transfer, *hormon* = to excite) are chemicals that are produced by an individual, released into the environment, and cause a change in another individual of the same species. More specifically, they start as liquids produced by specialized cells or glands and enter the environment as liquids or gasses. The excitement they cause in another individual is species specific. In honey bees, pheromones are involved in a complex and diverse array of behaviors: mating, swarming, brood rearing, egg-laying suppression of worker bees, and more.

Pheromone systems are common in many animals, especially in other insect species. They are found in mammals, and proposed human pheromones are linked to sexual attraction and arousal as well as mother-newborn interactions. Of the pheromone systems discovered to date, those present in the honey bee are among the most complex, if not the most complex humans have identified.

Queen Mandibular Pheromone

Information about the queen bee's pheromones is growing and ever-changing, and initially I will concentrate on the pheromones produced by the mandibular glands of the queen. The *queen mandibular pheromone* (QMP) is often called the *queen substance*. The term "queen substance" can be taken back to Dr. Colin Butler's proposal of such a substance in 1954, and it's identification in 1961 as the chemical 9-oxo-2-decenoic acid, commonly called 9-ODA. This chemical was isolated from the mandibular glands of queen bees. These glands are connected by a duct that connects to the mandibles of the queen. There are two glands, one for each mandible.

The mandibular gland secretions are not limited to one chemical molecule. In fact, 17 different compounds have been isolated from the mandibular glands that have been shown to provide some level of excitement in other bees. When Dr. Dewey Caron and I prepared his textbook *Honey Bee Biology and Beekeeping* (Wicwas Press 1999), he listed five molecules as the "most important". These five molecules work together, and the removal of any of them cuts the level of 'excitement' by half. The first three molecules are acids, and the last two are aromatic compounds:

9-ODA	9-oxodec-2-onoic acid
9-HDA	cis-9-hydroxydec-2-enoic acid
9-HDA	trans-9-hydroxydec-2-enoic acid
HOB	methyl-p-hydroxybenzoate
HVA	4-hydroxy-3-methoxyphenylethanol

Bees in the retinue lick, feed, clean and attend to the queen.

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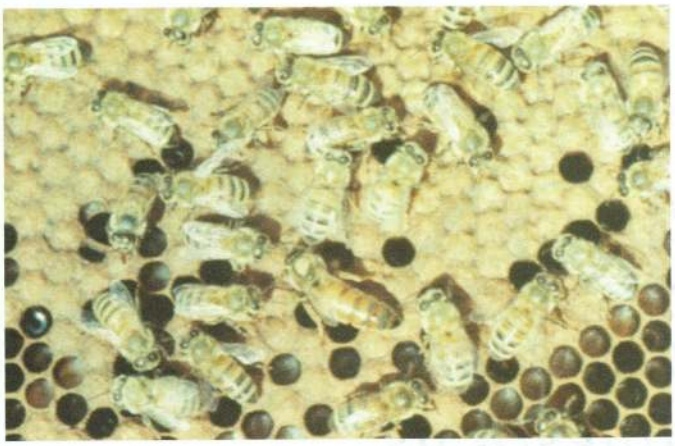

Getting ready to feed the queen.

These molecules, and their role as pheromones, were discovered in a series of research projects conducted since 1961. Importantly, not one of these molecules works alone (no better than the solvent control) but always as part of a blend of pheromones.

Queen Retinue Pheromone

A laying queen honey bee apparently performs two major functions in the colony. Ideally, she will oviposit about 200,000 eggs in worker and drone cells over the year, thus serving as the biological mother of the hive, passing her genes to her daughter workers and queens and to her drone sons. Secondly, she is the producer of pheromones from various glands and cells on her body. These pheromones allow her to perform many of her queenly duties in the colony.

In 2003, a great deal of information was added to our knowledge of queen pheromones in a paper titled *New components of honey bee (*Apis mellifera *L.) queen retinue pheromone* by Christopher Keeling, Keith Slessor, Heather Higo and Mark Winston of Simon Fraser University and published by PNAS. In this paper we transition from the idea of *mandibular* pheromone to *queen retinue* pheromone, which allows other molecules from the queen's body to contribute to the behavior we see in bees that visit the queen, groom her, and receive her pheromone.

Most beekeepers have watched the queen surrounded by her retinue of nurse worker bees. This occurs when the queen is resting from egg laying; during active egg-laying the retinue is non-existent or poorly defined. The bees in the retinue are not the same, but constantly changing. Some bees only make contact with the queen with their antennae. Apparently, enough of the queen pheromone is transmitted by antennal contact that the worker bee can walk through the hive with an excited level of pheromone on her body. By doing this, she lets the rest of the bees biologically "know" – via trace amounts of queen pheromone – that the queen is alive and well in the hive.

Other retinue bees lick the queen (see photo) to obtain pheromone. Other bees feed the queen while others remove her feces. The queen rectum produces pheromone, and pheromone-loaded feces is found in other organisms, (including the naked mole rats of Africa, which have queen moles which control daughters from breeding by using pheromones found in her feces).

An earlier research team at Simon Fraser University had found that the mandibular glands of a mated, laying queen contained a mixture of these amounts of pheromone, and defined this mixture as a *queen equivalent* (Qeq).

Chemical	Amount
9-ODA	~200 micrograms
9-HAD (both forms)	80 micrograms
HOB	20 micrograms
HVA	2 micrograms

A queen secretes one Qeq every 24 hours. She moves the pheromone to other parts of her body by her own self-grooming, and by worker grooming. Virgin queens produce significantly less 9-HAD, HOB and undetectable amounts of HVA; as a result, they are much less attractive to worker bees than mated queens. This provides us with useful information about the relative freedom virgin queens receive until they are mated. However, virgin queens are not completely free of pheromone, and they must be treated as a queen whenever introduced to a colony. In other words, they cannot be dropped into a colony without introduction, in spite of a number of noted authors who have suggested doing this as a means of queen introduction.

The next step in this story is the discovery that different colonies had a genetically controlled variation in their response to QMP. The Simon Fraser team found that the variation was not affected by the queen's QMP production, the worker's age, the worker's fostering colony, and the dose used in the bioassay. The researchers realized that anytime you discover a genetic component providing variation in a behavior for a trait, you can select for or away for that trait, and that is what they did. Two strains were developed from a two-way selection technique resulting in colonies with a high QMP response and other colonies with a low QMP response. The difference between these two strains was measured by a factor of nine; the high strains were nine times more excited by the QMP than the low strains when evaluated by the same testing methods.

Because colonies with a low QMP response still treated their queen normally, it was suggested that there were other molecules produced by the queen which added to the queen retinue response by worker bees. After looking at the tergite glands (located between the plates on the top of the queen's abdomen) Keith Slessor and his research team concluded that the pheromonal activity was due to translocated head pheromone and not a pheromone secreted by the tergite gland. This went against the popular opinion that the tergite glands were the source of the remaining pheromonal activity of the queen bee; this is expressed in John Free's summary of bee pheromones (*Pheromones of the Social Bees*, Cornell University Press, 1988), and the work of other researchers.

Keeling et al. then looked for pheromones produced in the head of the queen bee, but not by the mandibular glands. His research is very detailed and I am not going to

repeat his work here (a web-link is listed at the end of the article). It involves the use of pseudoqueens in a bioassay, queens which responded strongly to a queen extract and poorly to synthetic QMP (from PheroTech), analysis of extracts using gas chromatograph, liquid chromatography, dissection of queen and worker bees for the body section containing the greatest retinue activity and analysis of the bee blood (hemolymph) using gas chromatography-mass spectomography.

Although they did not give up their identify easily, Keeling identified four new compounds. None of them worked alone, but always as part of a synergistic interaction with QMP to excite worker bees. The resulting nine chemicals do not combine to provide the same retinue response of queen extract at higher doses, suggesting that there are still additional molecules to locate and identify.

The new molecules are:

Methyl oleate	Methyl (Z)-octadec-9-enoate
Coniferyl alcohol	(E)-3-(hydroxy-3-methoeytphenyl)pproop-2-en-1-ol
Hexadecane-1-ol	
Linolenic acid	(Z9,Z12,Z15)-octadexa-9,12,15-trienoic acid

Keeler et al. successfully selected colonies that had a low response to QMP and a high response to queen extract response. These selected colonies varied in their level of excitement to the new components. "*For example, some colonies could be classified as low or high MO (methyl oleate) responders.*" Other key observations include:

- Mated queens had more methyl oleate than virgin queens, but both had MO throughout their bodies, including the hemolymph (blood)
- Coniferyl alcohol was found in the mandibular glands of mated queens but not virgin queens.
- Coniferyl alcohol was not found earlier because it is very sensitive to light in methanol.
- Hexadecane-1-ol was located in the Dufour's gland and the cephalic labial gland of mated queens. In virgin queens it was found primarily in the abdomen.
- Linolenic acid was found in all three sections of the queen's body, but primarily in the thorax and abdomen.
- Because these pheromones arise from different glands in the queen's body, their production may be influenced by the metabolism of each gland. *"...other glandular components may change significantly when a mated queen begins to fail and starts laying unfertilized eggs. Because the retinue pheromone is used to facilitate the dispersion of other queen signals throughout the colony, changes to the retinue attraction may influence such things as queen rearing, swarming and worker reproduction. Further study of each gland involved is needed to understand its role in the queen."*
- Queens contained several aliphatic esters previously found on honey bee brood. These brood esters have a profound effect on colony function. *"Thus the queen seems to act in concert with her brood to modulate the behavior and physiology of worker bees, the retinue pheromone, which elicits the workers to lick, antennate, and groom their queen, entices workers to pick up these other control sociochemicals from their queen."*
- *"During this study, no colony was ever found that had a retinue response significantly higher for QMP than the queen extract, suggesting that queen recognition cues may only increase, and not inhibit, retinue response."*

Keeling et al. conclude with the following: "*Our study has increased the total number of components known to be involved in retinue attraction to nine, originating from several glandular sources. This is the most complex pheromone system known for a single behavior in any organism and provides an example of the complexity to be expected for pheromone communications in other social insects. Not all of the components involved need to be unique to the queen because synergy and the contextual presentations are critical to elicit a response. This emphasizes the necessity of a bioassay-guided approach to pheromone isolation and identification. QMP should no longer be considered a distinct retinue pheromone but rather a larger portion of a complex queen retinue pheromone. In addition to QMP and the four new components, the queen also possesses compounds that, although not essential for retinue attraction, may be crucial for the control she exerts on her colony.*"

As beekeepers, we cannot help but appreciate the work of the Simon Fraser University researchers as very important effort to increase our ability to replace queens and manage bees in multiple queen systems. Of course, many beekeepers dream of using some yet-discovered pheromone that allows them to coat the queen and drop her into the hive. We are not there yet, and it may never be that easy. But the better beekeepers understand the role of pheromones produced by our queens, the better we will be able to manage colonies using the information available to us today.

Further Reading

This is strong stuff, but I suggest you will greatly increase your beekeeping knowledge if you stick with it. I am not a chemist, but survived organic chemistry in college yet much of this work makes my head spin a bit, just trying to keep everything straight. It helped me to review the information as it was discovered, and I strongly recommend these books, from your local library or your local new/used bee book seller. Please email me at LJConnor@aol.com if you need help finding these publications. BC

References

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