

What's the essence of royalty – one keto group?

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A honey bee colony consists of a single queen, tens of thousands of nearly sterile female workers and usually a few hundred drones. The presence of the queen inhibits rearing of new queens, stimulates foraging and interaction of workers with the queen and, (along with the queen's brood), inhibits the development of worker ovaries. Most or all of these effects of the queen on the workers are mediated through primer pheromones secreted by the queen. An important component of the queen's pheromone blend is 9-keto-(E) 2-decenoic acid (9-ODA). Workers also produce related substances which appear to function as nutrients and food preservatives. A dominant component of the worker blend is a diacid which is made from a precursor molecule hydroxylated at the ω

carbon atom rather than at the $\omega-1$ carbon atom. The $\omega-1$ precursor is used by the queen which leads to the formation of a keto acid. One might say that the fundamental difference between a queen and worker, the essence of royalty is therefore, one keto group! The recently-elucidated caste-specific biosynthetic pathway for the production of these pheromones permits two other speculations. One is that workers can be thought of as being closer to the ancestral solitary condition and that queens can be thought of as a derived invention of sociality. The other is that, compared to non-social species, social insects are especially predisposed to evolve novel structures and characters as exemplified by the queen for example, through the process of evolution of gene duplication.

Honey bees

WILLIAM Morton Wheeler¹ says of the honey bee: 'Its sustained flight, its powerful sting, its intimacy with flowers and avoidance of all unwholesome things, the

attachment of the workers to the queen – regarded throughout antiquity as a king – its singular swarming habits and its astonishing industry in collecting and storing honey and skill in making wax, two unique substances of great value to man, but of mysterious origin,

made it a divine being, a prime favourite of the Gods, that had somehow survived from the golden age or had voluntarily escaped from the garden of Eden with poor, fallen man for the purpose of sweetening his bitter lot.' Today one might add, 'and also for the purpose of providing him with a model system to study everything he wishes to know about animals'. Honey bees have served as an excellent model system in animal behaviour, sociobiology, physiology, genetics and biochemistry. Above all the discovery by Karl von Frisch², of colour vision in the honey bee and of its ability to use ultraviolet as another colour and see patterns in flowers that we cannot, and his elucidation of the dance language of the honey bee have created in us a new respect for animals. In the words of J. L. Gould, 'The lesson is a melancholy one. We are blind to our own blindness and must not try to read our disabilities into the rest of the animal kingdom.' Now honey bee queens and workers are on the threshold of teaching us the essence of royalty and the lesson is an even more melancholy one – the answer seems to be, one keto group!

The queen pheromone

Honey bees live in populous colonies consisting of tens of thousands of sterile workers, a few hundred drones and a single fertile female, the queen. The queen affects the workers in several ways^{3,4}. The presence or absence of the queen is detected by the workers in a matter of minutes. The presence of the queen normally prevents the workers from rearing new queens. Also, the presence of the queen and her brood inhibits the development of the worker's ovaries; in the event of the death of the queen, however, workers do develop their ovaries and lay small numbers of unfertilized haploid eggs. The queen is always surrounded by an ever-changing retinue of about 10 workers at a time who feed and lick her. Most or all these effects of the queen on the workers are mediated by pheromones secreted largely from a pair of mandibular glands on either side of her head. Pheromones are chemicals, usually but not always volatile, secreted from exocrine glands of animals which serve to elicit behavioural or physiological responses in conspecifics and thus serve as chemical messengers⁵. The best-known pheromones such as bombykol, the sex pheromone of the silk moth that helps the male find his mate with incredible sensitivity, are seen to release specific and instantaneous behavioural responses and hence are termed *releaser* pheromones. The honey bee queen pheromone on the other hand, has more complex and fundamental effects on workers, including inhibition of their ovarian development and is hence termed a *primer* pheromone. The queen bee pheromone (which acts both as a releaser and primer pheromone)⁶ is a blend of several chemicals not all of which may yet be known.

However Mark L. Winston and Keith N. Slessor of Simon Fraser University in British Columbia, Canada, have succeeded in identifying five of the most essential components of the queen pheromone which together elicit most of the important behavioural responses expected from the workers⁷. One queen equivalent of what I will call the Winston–Slessor blend (although they prefer to call it QMP, for queen mandibular pheromone, to emphasize their team work involving a large number of other people), consists of about 200 µg of 9-keto-(E)-2-decenoic acid (9-ODA), about 80 µg of 9-hydroxy-(E)-2-decenoic acid (9-HDA), of which about 56 µg is the (–) optical isomer and about 24 µg of the (+) optical isomer, about 20 µg of methyl *p*-hydroxybenzoate (HOB) and about 2 µg of 4-hydroxy-3-methoxyphenylethanol (HVA) (Figure 1). The latter two aromatic compounds are minor and indeed, somewhat unexpected components. On the other hand the aliphatic 9-ODA and 9-HDA are the major components whose involvement in the honey bee queen pheromone has been known for a long time.

The Winston–Slessor blend

The Winston–Slessor blend elicits a clear-cut retinue response, indeed that is how it all started when Kaminski, Slessor and Winston⁸ noticed that stray worker bees formed a retinue around a glass vial containing a crude extract of the queen mandibular gland. Much of their subsequent work is based on a bioassay based on the retinue response that workers so readily show to the chemicals *sans* the queen bee. The blend also mimics the queen's ability to inhibit queen rearing by the workers. In the event of the death of the queen, workers resort to emergency queen rearing by enlarging some of the cells containing young (< 3 days old) larvae and feeding the chosen larvae with 'royal jelly' and thus channeling them into a developmental pathway leading to the formation of queens. When workers in queen-less colonies were given one queen equivalent of the blend per day, emergency queen rearing was almost completely inhibited. Although the blend elicited other expected responses such as inhibition of swarming, it did not inhibit worker ovarian development, which is therefore thought to be a function of a different primer pheromone not included in the Winston–Slessor blend (and of the brood). An unexpected effect of the blend was its stimulation of pollen foraging and brood rearing. By spraying crops with the blend and showing that more bees visit the crops and whose yield then increases due to better pollination, Winston and his colleagues have demonstrated a promising commercial application of their basic research – what a sigh of relief in this day when there is so much pressure to make economic sense of all scientific research!

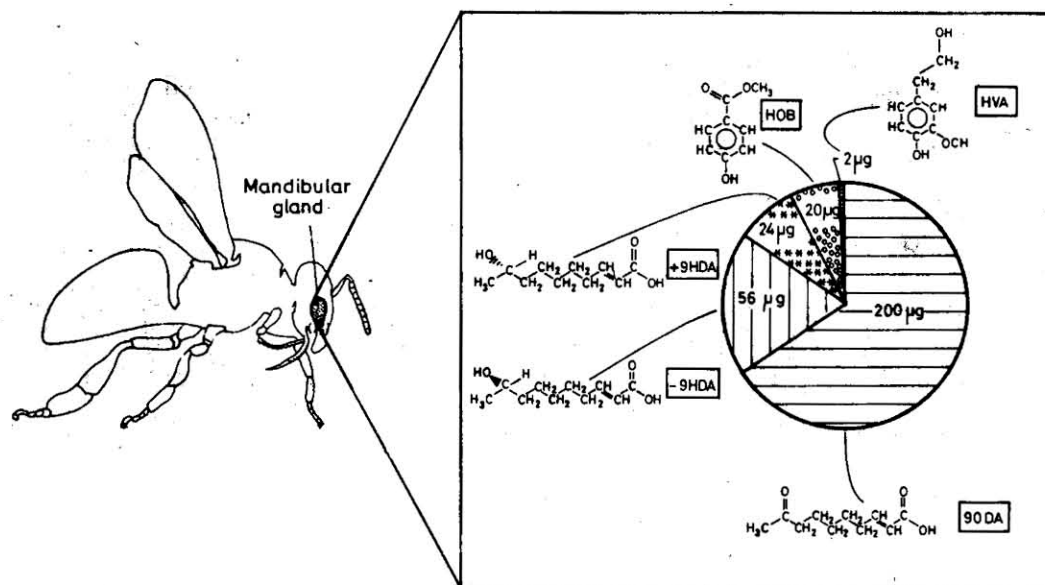


Figure 1. The Winston-Slessor blend of honeybee queen pheromone containing well-defined amounts of five of the most important components of the mandibular gland secretion, which elicits most of the responses expected from workers. See text for expansion of the names of the components.

Queen-worker dichotomy

Having made some economic sense, we deserve the freedom to turn our attention once again to a whole new intellectually challenging question. Perhaps the most fascinating aspect of honey bee colonies is the differentiation of the bees into a sterile worker caste and a fertile queen caste. The question that stems from this observation relates to the possible differences between queens and workers in their pheromone blends and the mechanism of the origin of these differences. These are the questions that Plettner *et al.*⁹ address in a recent path-breaking paper. Workers too produce mandibular gland secretions that are added to the brood food and may serve as preservatives and nutrients. Instead of the two major components of the queen's secretions namely, 9-ODA and 9-HDA, workers secrete acids hydroxylated at the 10th or ω -carbon atom rather than the 9th or $\omega-1$ carbon atom as in the case of the queen's acids. Instead of the queen's 9-HDA, workers secrete 10-hydroxy-(E)-2-decanoic acid (10-HDA) and instead of the queen's 9-ODA, workers secrete the diacid derived from their 10-HDA. In other words, queens and workers differ essentially only in the position of the carbon atom that is hydroxylated. But how does this difference arise? As a result of a series of experiments involving analysis of the fate of deuterated test compounds applied to excised queen and worker mandibular glands, using gas chromatography-mass spectrometry (GC-MS), Plettner *et al.*⁹ have proposed the following caste-specific, bifurcated three step biosynthetic pathway for the production of these compounds (Figure 2).

A caste-specific pheromone biosynthetic pathway

The starting point is stearic acid, a very common, 18 carbon, straight chain saturated intermediate of lipid oxidation¹⁰. In the first step of the proposed pheromone biosynthetic pathway, functionalization is achieved by the addition of a hydroxyl group on either the 18th (ω) or the 17th ($\omega-1$) carbon atom. This functionalization which foreshadows the queen-worker differences depending on whether it happens at the ω or the $\omega-1$ carbon atom is, however, itself not caste-specific; both ω and $\omega-1$ functionalizations occur in both castes to about the same extent. In the second step, the 18-carbon hydroxy acids are shortened to give 10-HDA and 9-HDA by the standard chain-shortening cycles of β oxidation that normally occur during fatty acid metabolism. It is the β oxidation step that is caste-specific – queens preferentially channel the $\omega-1$ compounds and workers preferentially channel the ω compounds into the β oxidation pathway. In the final step, oxidation of the ω or $\omega-1$ hydroxy group that was added in the first step, results in the formation of the diacid in the case of workers and the keto acid in the case of queens. The evidence for every feature of the proposed pathway is clear and convincing. Labelled stearic acid is incorporated into the final products but labelled palmitic and decanoic acids are not. There is no isomerization between 10-HDA and 9-HDA. Both the ω and $\omega-1$ functionalized hydroxy acids are detected to the same extent

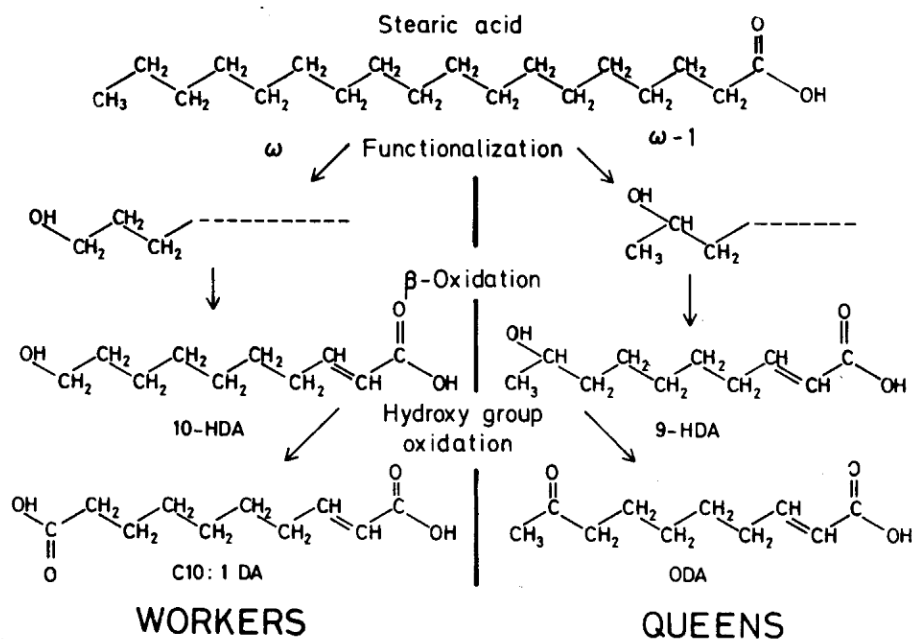


Figure 2. The caste-specific, three-step, bifurcated pathway for the biosynthesis of queen and worker pheromones, as proposed by Plettner *et al.*⁹

in both castes but the subsequent steps are entirely caste-specific. That functionalization precedes β oxidation is evident from the fact that hydroxy acids with more than 10 carbons accumulate when an inhibitor of β oxidation, 2-fluorostearic acid is added to the reaction mixture. Thus both workers and queens add hydroxy groups to either the ω or the $\omega-1$ carbon atom but workers then preferentially convert the ω functionalized compound to produce 10-HDA and the corresponding diacid while the queens preferentially convert the $\omega-1$ functionalized compound to produce 9-HDA and the corresponding keto acid, 9-ODA. Since 9-ODA is the major component of the queen pheromone, I am tempted to dramatize and say that the essence of royalty is just one keto group!

Plettner *et al.*, however, merely conclude modestly from these remarkable findings that 'These results demonstrate how, in a social insect, caste-determined biosynthesis of isomeric compounds can produce markedly different glandular blends that are responsible for many functional differences between queens and workers.' In addition to the dramatization of the essence of royalty as one keto group, I believe that these results permit two other speculations of the considerable significance for our understanding of social evolution.

A chicken and egg problem

The first speculation concerns the usual chicken and egg problem – who came first, the queen or the worker? On the one hand, queens in social insect species can be thought of as being comparable to the undifferentiated

(into queen or worker) adult insects in their solitary ancestors (or, equivalently, in other extant solitary taxa) and the workers can be thought of as being a new invention of sociality. After all, adults in solitary species are all potentially capable of reproducing and it is the character of being sterile and merely working to rear another individual's brood, that is a novel feature of social insects. On the other hand, workers in social species may be thought of as being comparable to their solitary ancestors or extant solitary counterparts and the queens can be thought of as an invention of sociality. After all, adults in solitary species are all capable of nest building, foraging and brood rearing and it is the character of inhibiting reproduction of conspecifics and attempting to become the sole reproductive in a group, at the cost of losing foraging and brood-rearing abilities altogether if necessary, that is a novel feature of social insects. A reasonable solution to this conundrum is to compromise and think of the solitary insects as queen and worker combined because each individual is capable of reproduction as well as nest building, foraging and brood care. And this is a largely correct solution because both queens and workers, at least in the advanced social species, are considerably modified and exaggerated in their respective roles compared to solitary insects. Nevertheless, I believe that, if and when possible, we should try to make an objective assessment of whether queens are ancestral and workers are derived or whether workers are ancestral and queens are derived. I will argue that the pheromone biosynthetic pathway elucidated by Plettner *et al.*⁹, provide one such opportunity.

I hypothesize that the pheromone biosynthetic pathway employed by the workers deviates relatively little from the typical lipid metabolism pathway and is perhaps simply adopted from there. The diacid they make can relatively easily be channeled into an energy-generating role and its degradation products can be profitably fed into the Krebs's cycle. On the other hand, I speculate that the pheromone biosynthetic pathway of the queens is quite a deviation from the standard lipid metabolism pathway. In particular the keto acid is not something one would expect if energy generation is their goal. The expense involved in further breaking down the keto acid makes it a poor candidate to be fed into the Krebs's cycle. I therefore speculate that in the course of making their pheromones, the workers are doing more or less what any solitary insect would do anyway for generating energy from lipids and that their pheromone biosynthetic pathway is therefore the more ancestral one. Conversely, queens have considerably modified the ancestral lipid metabolism pathway in order to make a pheromone that has only lately (relatively speaking) become necessary. In order to do so, they are prepared to make an end product such as the keto acid which is energetically unwise but I argue that energy generation is not their motivation here. Surely they have other mechanisms of generating energy even from lipids. Even if their overall efficiency of generating energy from lipids is lower than that of workers, it does not matter that much because it is the foragers, not the queens, that have to fly great distances in search of food. The pheromone biosynthetic pathway of the queens appears therefore to be relatively more derived. One might also argue that the function of the worker pheromone namely, to act as a preservative and nutrient is also a more ancestral function, more likely to have been useful in the solitary condition. Conversely the function of the queen pheromone appears to be more derived as it fulfills a relatively more recent requirement and hence is unlikely to have been of much use in the ancestral solitary condition. Workers thus seem to use an ancestral biochemical pathway to make a product that may also have been required in the ancestral condition. And queens seem to be using a rather derived form of the biochemical pathway to make a product that has a rather derived function. At least in this limited context, workers seem to be ancestral and queens seem to be derived. This one context, important as it is, cannot be thought to have solved our general problem of who came first, the queen or the worker. It would be prudent, even necessary, to be on the look out for more opportunities to classify queens and workers as ancestral or derived. Indeed, a new and highly derived function of the worker pheromone may yet be discovered which may alter our conclusion. Thus, we may well come up with different conclusions each time and only the relative scores for 'ancestral' and 'derived' that queens and workers accu-

multate in the long run can help us solve this conundrum in any general sense. But I believe this is a good beginning.

The evolution of caste polymorphism

Yet another striking feature of the social insects, the highly social insects in particular, is the morphological differentiation of queens and workers which may sometimes reach such proportions that, if encountered separately, queens and workers may get classified as different species¹¹. The greatest intra-specific size variations have been recorded in the Asian ant *Pheidologeton diversus*, where some workers weigh 500 times and have a head width 10 times compared to other workers¹². Here the differentiation is not between queens and workers but between the so-called major workers and minor workers. Whether it is between queens and workers or between major and minor workers, these extreme degrees of intra-species, intra-sexual dimorphism require an explanation. The fact that no solitary species seem to match these levels of differentiation suggest that the explanation is linked to the social habit of these insects.

I have recently offered a speculation¹³⁻¹⁵ which was inspired by the idea of evolution by gene duplication first suggested by Haldane¹⁶ and Muller¹⁷ and elaborated and championed by Susumu Ohno¹⁸. The idea is that redundant, duplicate copies of genes can accumulate potentially lethal mutations without killing the organism and eventually can give rise to novel genes coding for novel structures via pathways that would be inaccessible to an individual with a single copy of the gene. I have argued that a very similar consequence will accrue to social insects although for a somewhat different reason. The evolution of altruistic sterile worker castes in the social insects was considered paradoxical until Hamilton proposed the theory of inclusive fitness¹⁹. Today it is common practice to recognize inclusive fitness as having two components, a direct component gained through production of offspring and an indirect component, gained through aiding close genetic relatives. Sterile worker castes are expected to gain fitness exclusively through the indirect component^{4,20,21} and in no other group is there a comparable level of dependence on the indirect component of inclusive fitness.

When some individuals in a species begin to rely on the indirect component of inclusive fitness while others continue to rely on the direct component, as workers and queens in social insects are expected to do, I have argued that different sets of genes in queens and workers will be liberated from previous epistatic constraints and become free to evolve in new directions, because the same individual no longer has to optimize both reproductive and non-reproductive functions. There is no gene duplication here in the conventional sense but the

consequence namely, liberation from previously existing constraints (due to the action of stabilizing selection) and the opportunity to diversify in different directions (through the action of directional selection), is similar. To put it simply, an individual can evolve into a 'super' egg layer if it does not also have to simultaneously be a good forager or it can evolve into a 'super' forager if it does not also have to simultaneously be a good egg layer.

I wish to speculate now that compared to solitary species, social insects are also in a better position to exploit the evolutionary advantages of conventional gene duplication. I have argued in the previous section that the function of the worker pheromone and the biochemical pathway involved in its production are relatively more ancestral and that the function of the queen pheromone and the biochemical pathway involved in its production are relatively more derived. If this is true, it is not difficult to see the tremendous advantage of conventional gene duplication in bringing about the derived condition from the ancestral one. It seems likely that the enzymes involved in the β oxidation step (Figure 2) give rise to specificity for substrates hydroxylated at the ω or $\omega-1$ positions. Imagine that the ancestor of the social insect species had a gene that coded for an enzyme which could deal only with the substrate that was hydroxylated at the ω position. The workers in the descendant social species can continue to use this gene and this enzyme to make worker pheromones which may perhaps have even been made by the ancestor. A duplication of the gene involved can permit the evolution of an alternate enzyme which can handle the substrate hydroxylated at the $\omega-1$ position. We know that such a substrate must already have been available because both kinds of hydroxylations occur to an equal extent in both queens and workers. The duplicated gene would now be free to evolve in new directions without reduced fitness due to the reduction in the efficiency of energy production through lipid metabolism. And new directional evolution can sometimes give rise to substances with such remarkable properties as those of the queen pheromone. A similar chance occurrence of such evolution could hardly have been utilized effectively by a solitary species. Because social insects set aside some individuals for the sole purpose of monopolizing reproduction and inhibiting and controlling all others, they are in a special position to exploit such a consequence of conventional gene duplication.

Evolutionary biologists have often found it useful to clearly distinguish between proximate physiological explanations and ultimate evolutionary explanations. Indeed, failure to make this distinction has sometimes led to unnecessary confusion as to what constitutes a valid answer to the question of why an animal does what it does. However it would be unfortunate if we permanently

delink the study of proximate mechanisms and ultimate evolutionary explanations²². The biochemical pathway for the caste-specific biosynthesis of pheromones elucidated by Plettner *et al.*⁹, constitutes an excellent illustration of how our understanding of the proximate and ultimate factors can mutually reinforce each other. It is high time, that evolutionary biologists became biochemists and vice versa!

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