

Organization of Insect Societies

FROM GENOME TO SOCIOCOMPLEXITY



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CHAPTER TEN



Fertility Signaling as a General Mechanism of Regulating Reproductive Division of Labor in Ants

CHRISTIAN PEETERS

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DIVISION OF LABOR is the essence of sociality in insects and its most striking manifestation is the sterility of almost all colony members. Unequal reproduction among relatives is associated with many conflicts, and recent studies have reconciled these with kin selection (Bourke and Franks 1995). In this chapter, we examine the essential role of communication in the resolution of conflicts over reproduction. Information exchange is indeed entangled with both cooperation and conflicts.

Social Hymenoptera exhibit a broad variety of colony patterns, ranging from small societies with nestmates having equivalent reproductive potentials (e.g., queenless ants, *Polistes* wasps) to huge societies with morphologically highly specialized queens and workers. Although similar conflicts transcend this heterogeneity, the mechanisms that regulate monogyny or queen supersedure, for example, are not the same at either ends of this spectrum. We will argue that seemingly dissimilar regulatory mechanisms rely all on just one category of olfactory information.

All insects are covered with cuticular hydrocarbons which act as a desiccation barrier. There are many distinct molecule types in each species (chain lengths from C23 to C37, with differing numbers and positions of double bonds or methyl-branches), and blends of cuticular hydrocarbons reflecting variations in age, reproductive physiology, and genotype among individuals. In social Hymenoptera, these cuticular differences encode information that seems instrumental in conflict resolution.

From the Past to the Present

Queen Pheromones: A Paradigm Shift

In this chapter we restrict the terms *queen* and *worker* to morphologically specialized adult females (Peeters and Crozier 1988), in contrast to morphologically undifferentiated *breeders* and *helpers* as in *Polistes*. Pheromones have long been known to be involved in the reproductive division of labor in ants and honey bees; unlike vertebrate societies, physical aggression is usually not needed to regulate reproductive skew. Experimental studies in many social insects have established that secretions from the queen are sufficient to mimic her presence (Passera 1984; Hölldobler and Bartz 1985; Fletcher and Ross 1985; Vargo 1998). It remains controversial whether these queen pheromones have a coercive effect on the receivers (i.e., direct inhibition of ovarian physiology) or whether they simply carry information used by receivers to behave adaptively (Seeley 1985; Keller and Nonacs 1993). Several lines of evidence make chemical inhibition unlikely. Its physiological basis has never been made explicit; for example, is the queen pheromone absorbed by the corpora allata or the ovaries, instead of being detected by the antennae? In addition, there has been no demonstration that receivers ever behave against their own interest, which would support inhibition. Moreover, pheromonal manipulation would be evolutionarily unstable because of a likely "arms race." Instead, self-restraint by receivers brings benefits as long as the reproductive is related and sufficiently productive (Keller and Nonacs 1993). Accordingly, queen pheromones may simply carry information about current fertility.

The sources of queen pheromones have largely remained an enigma, with the exception of *Apis* in which the mandibular, tergal, and Dufour's glands are known to be implicated (Winston and Slessor 1998). In ant queens, the efforts to investigate various exocrine glands have had little progress. In *Solenopsis invicta*, poison gland secretions of queens inhibit alate gynes from shedding their wings and developing their ovaries, but queens without poison sacs caused a similar primer effect. Vargo and Hulseley (2000) hinted that another relevant pheromone could be distributed over the body. In the ponerine ant *Megaponera foetens*, a queen is highly attractive to the workers, and her pheromone may originate from a thick glandular epithelium lining her entire body (Hölldobler, Peeters, and Obermayer 1994). In *Bombus terrestris*, where there is no caste dimorphism,

a cuticular wash from reproductives caused inhibition of the helpers' ovaries, unlike the extracts from five exocrine glands (Bloch and Hefetz 1999). In hindsight, experimental results from different social insects are not incompatible with the queen pheromone being located on the cuticle, but researchers tend to explain such results as contamination from exocrine glands.

Dominance Behaviors and Recognition

A large number of Hymenopteran societies consist of female adults with equivalent reproductive potentials. In the absence of morphological castes, aggressive interactions lead to the formation of dominance hierarchies and only the alpha individual (or several high-ranking individuals in polygynous species) has active ovaries and reproduces. These dominance interactions are highly directed, showing that hierarchy members can be recognized. In *Polistes* wasps, aggression and the chemical communication of status were shown to be separate phenomena (West-Eberhard 1977; Downing and Jeanne 1985). Similarly, in *Leptothorax* ants competing queens can recognize individual ovarian status and this affects their pattern of aggression and acceptance (Ortius and Heinze 1999). Chemical signaling of ovarian activity had indeed been inferred by several authors (e.g., Wilson 1971; Ratnieks 1988; Visscher and Dukas 1995), but there was no direct evidence for it. Behavioral observations of dominance interactions in *Harpegnathos saltator* hinted that subordinates detect the onset of ovarian activity in gamergates (mated reproductive workers) by antennating their cuticle (Liebig 1998). Researchers of the wasp *Polistes dominulus* and the bumblebee *Bombus hypnorum* found differences in cuticular hydrocarbons that were associated with ovarian activity (Bonavita-Cougourdan et al. 1991; Ayasse et al. 1995)

Such considerations aside, an investigation of cuticular hydrocarbons and their function started in the queenless ant *Dinoponera quadricaps* because of a conspicuous aggressive behavior. During hierarchy formation, the alpha worker bites an antenna of a subordinate and rubs it against the dorsal region of her gaster (Monnin and Peeters 1999). At that time, solid-phase microextraction (SPME) was being developed for the analysis of pesticide residues in water, and following a suggestion by Robin Crewe, Monnin, Malosse, and Peeters (1998) rubbed an SPME fiber against *Dinoponera's* gaster in the same way that the subordinates' antennae are

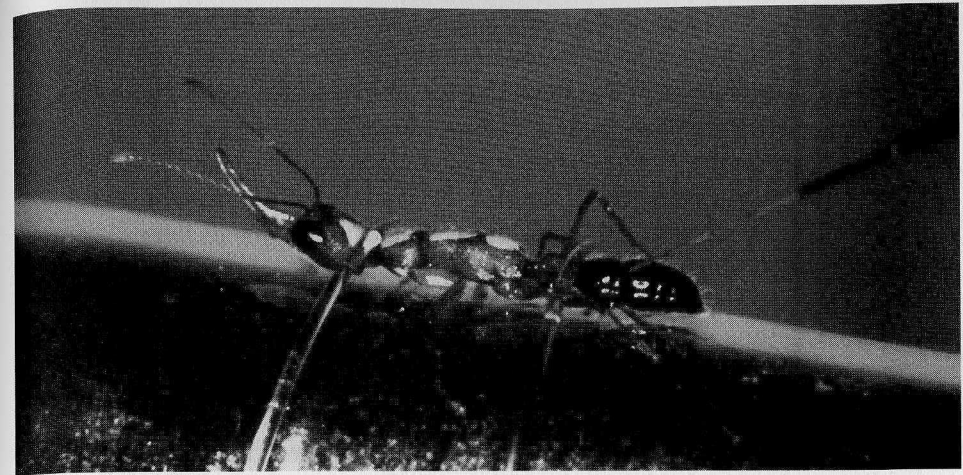


Figure 10.1. Use of SPME to sample cuticular hydrocarbons from a *Harpegnathos* worker. Nylon strings are used to immobilize the ant, and the fiber is gently rubbed over the gaster.

rubbed. Gas chromatography revealed that the alpha worker's blend of cuticular hydrocarbons differed from that of infertile nestmates, with an alkene of 31 carbon atoms present in high proportions. If the beta worker (number two in hierarchy) replaces alpha, her ovarian activity increases; SPME allowed the analysis of live beta workers before as well as after such replacement and the proportion of the alkene increased concomitantly (Peeters, Monnin, and Malosse 1999). The same method (Figure 10.1) used in a study of *Harpegnathos saltator* showed that workers simultaneously shift their cuticular profile when they differentiate into gamergates (Liebig et al. 2000). In this species the whole profile changes; longer molecules gradually increase in proportion while shorter molecules decrease over a period of 118 days. Prior to SPME, destructive extraction with solvents had prevented monitoring individual ants at multiple times during their adult life.

Cuticular Hydrocarbons Are Reliable Markers of Reproductive Physiology

A link between oogenesis and the synthesis of cuticular hydrocarbons is found in many solitary insects (e.g., cockroaches, Diptera) and probably

involves gonadotropic hormones (Dillwith, Adams, and Blomquist 1983; Tralabalon et al. 1990; Wicker and Jallon 1995). In both *Drosophila* and *Caliphora* some of these hydrocarbons function as sex pheromones (e.g., Fer-veur, Cobb, and Jallon 1989). Cuticular hydrocarbons also represent a sex pheromone in the cricket *Gryllus bimaculatus* (Tregenza and Weddell 1997), while in cockroaches they may reveal dominance status of males (Roux et al. 2002). The display of information about fertility makes cuticular hydrocarbons appropriate for communication during courtship behavior in solitary insects. During the evolution of social species this information appears to have been co-opted for new functions relating to cooperation and the regulation of reproductive conflict. To date, intracolony variations in cuticular hydrocarbons have been studied in at least 26 genera of ants, wasps, and bees (Monnin 2006; Liebig and Peeters, in prep.). In the following paragraphs the results of these studies are summarized for various reproductive contexts in which communication is needed about either the onset or decline in egg-laying activity.

Behavioral Regulation and Recognition of Hierarchy Members

In queenless ants, several high-ranking workers compete aggressively to become the gamergate(s). Once behavioral differentiation has happened and ovarian activity begins, however, aggression declines and is replaced by chemical signaling (e.g., Monnin and Peeters 1999; Sledge, Boscaro, and Turillazzi 2001; Cuvillier-Hot et al. 2002; Cuvillier-Hot et al. 2004). Fertility has a striking effect on hydrocarbon profiles in all species investigated so far. In *Diacamma ceylonense*, C25 and C27 monomethyls occur in high proportions in egg-layers (Cuvillier-Hot et al. 2001). In *Streblognathus peetersi*, the cuticular profiles of gamergates are very distinct compared to that of infertile nestmates (callows and foragers); high-ranking workers are intermediate between the two, while newly differentiated alphas (i.e., oogenesis has just begun) resemble gamergates (Cuvillier-Hot et al. 2004). Such alphas are recognized by nestmates within 1 to 2 days of differentiation, even though they can only begin to lay eggs 30 days later (Cuvillier-Hot, Renault, and Peeters 2005). In *Gnamptogenys striatula*, fertile workers have longer-chained hydrocarbons on their cuticle compared to infertile nestmates (Lommelen et al. 2006). In multiple-foundress colonies of the wasp *Polistes dominulus*, alpha individuals (who monopolize oviposition) and subordinates initially have the same proportions of

cuticular hydrocarbons, but these proportions become distinct by the time the first adults start to emerge in a colony (Sledge, Boscaro, and Turillazzi 2001).

Queen Recognition and Worker Sterility

Ants exhibit a large spectrum in the degree of dimorphism (e.g., body size, number of ovarioles) between the castes. In *Harpegnathos saltator*, queens and workers are morphologically similar (except for wings) and gamergates reproduce once the founding queen has died. In these two categories of egg-layers, the proportions of cuticular hydrocarbons change in a similar way with the onset of ovarian activity, while young virgin queens resemble infertile workers (Liebig et al. 2000). Thus the hydrocarbons are not related to morphological caste but to reproductive physiology. Caste dimorphism is more marked in the bulldog ant *Myrmecia gulosa* and workers cannot mate. Fertile queens exhibit high proportions of a C25 alkene (Figure 10.2), while virgin workers lay reproductive eggs once orphaned and their cuticular profiles shift to resemble that of queens (Dietermann et al. 2003). Similarly in *Pachycondyla cf. inversa*, the cuticular profile of egg-laying workers differs from that of sterile nestmates but resembles the queen's (Heinze, Stengl, and Sledge 2002).

Variations in cuticular hydrocarbons are also found in phylogenetically derived ants with large caste dimorphism. In *Camponotus floridanus* (Formicidae), 10 out of 34 compounds in the cuticular profile of a highly fertile queen are absent on the workers' cuticle (Endler et al. 2004). These ten compounds represent more than 60% of the total amount of cuticular hydrocarbons in some queens (Endler, Liebig, and Hölldobler 2006). *Linepithema humile* (Dolichoderinae) presents another example of strong differences between workers and mated egg-laying queens. More than 50% of the total amount of the profile is specific to the fertile queens (de Biseau et al. 2004); 9 out of 33 identified compounds underlie this difference. In contrast, the cuticular hydrocarbon pattern of young virgin non-laying queens overlaps almost completely with that of workers. In this species workers lack ovaries and are thus completely sterile—yet they need information about the fertility of their queens to regulate polygyny. In the weakly polygynous *Formica fusca*, reproductive output is reflected by cuticular blends, and the more eggs a queen produces the more attention she receives from workers (Hannonen et al. 2002). Forsyth (1980) had

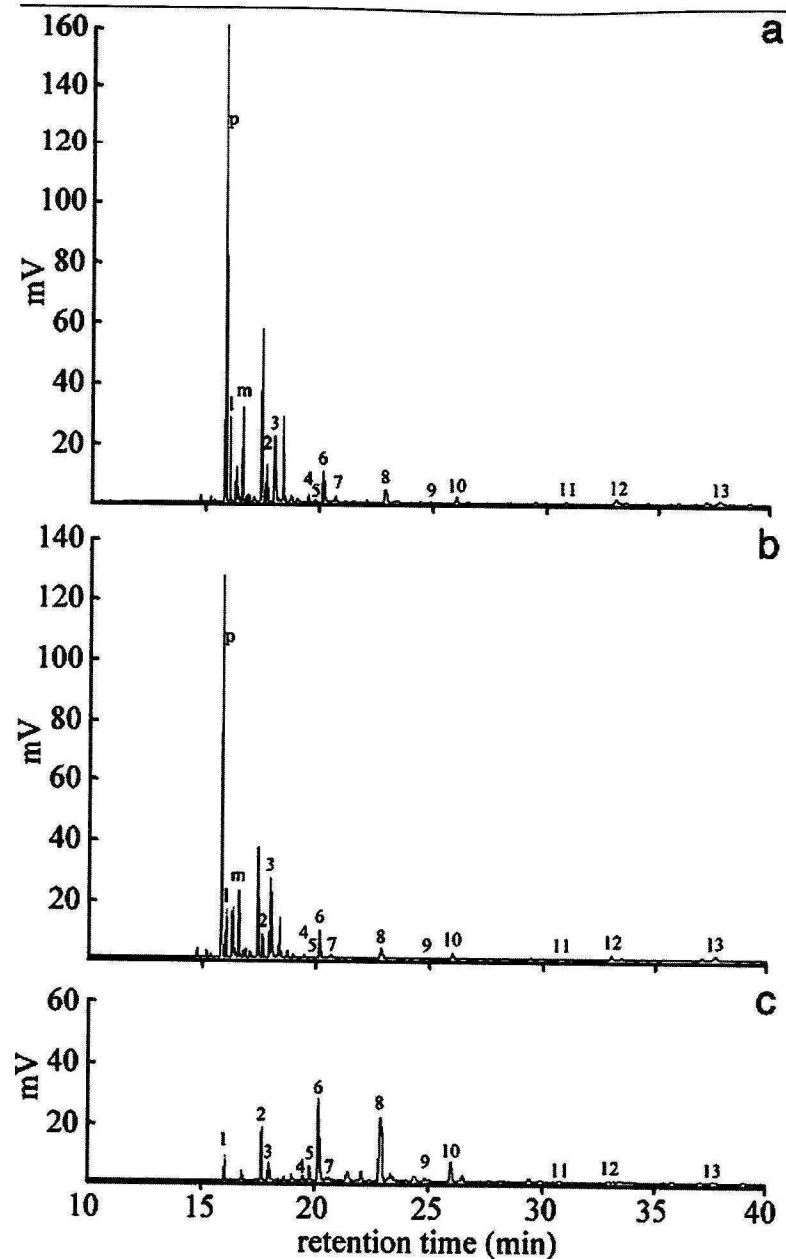


Figure 10.2. Gas chromatograms showing differences in the cuticular hydrocarbons of (a) fertile queen, (b) orphaned worker laying reproductive eggs, and (c) infertile worker of *Myrmecia gulosa* (from Dietemann et al. 2003). Numbers indicate the same compounds across categories. Compounds p and m are specific to reproductive individuals.

evoked the ability of Hymenopteran workers to assess differences in queen productivity during the execution of supernumerary queens. Cuticular differences among queens could serve as a proximate mechanism for the workers to determine which queens are less fertile and can be executed. Workers of *Lasius niger* (Sommer and Hölldobler 1995) and *Solenopsis invicta* (Fletcher and Blum 1983) favor the most fertile queen following pleometrosis (colony founding by multiple queens).

Worker Policing and Recognition of Selfish Egglayers

Several studies showed that ant workers can detect differences in levels of ovarian activity by olfaction. In *Gnamptogenys menadensis*, infertile workers discriminated between virgin workers laying male eggs and gamergates with more active ovaries; other workers laying trophic eggs were ignored (Gobin, Billen, and Peeters 1999). In *Harpegnathos saltator*, infertile workers attacked newly ovipositing gamergates in the presence of gamergates with more active ovaries (Liebig, Peeters, and Hölldobler 1999). The profiles of these newly ovipositing workers were already distinct at the time when they were attacked (Liebig et al. 2000). For *Myrmecia gulosa*, an experimental situation was created where infertile workers could interact with both their queen and workers that started to develop their ovaries; some infertile workers immobilized many of the latter by holding on to their antennae, legs, or body (Dietemann, Liebig, et al. 2005). SPME was used to show a correlation between the change in the victims' cuticular hydrocarbons and the likelihood of immobilization. In *Platythyrea punctata*, worker policing was also correlated with changes in cuticular profiles of new reproductives (Hartmann et al. 2005).

In queenless ants, worker policing has an additional crucial function during the selection of gamergates (Monnin and Ratnieks 2001). High-ranking workers can attempt to replace a gamergate prematurely, while low-ranking workers benefit from preventing the overthrow of a gamergate (usually their mother) as long as she is sufficiently productive. Reliable assessment of fertility is needed to establish the optimal time to replace a senescent gamergate and to identify premature challengers. In *Streblognathus peetersi*, policing workers played a crucial role during the replacement of gamergates with experimentally reduced fertility; they immobilized the manipulated gamergates, thus allowing supersedure by a high-ranking worker. Direct aggression was not observed between the

high-ranking individuals and the gamergate (Cuvillier-Hot, Lenoir, and Peeters 2004). Reduced fertility was accompanied by a predictable shift in the cuticular hydrocarbon profile of the gamergate, and an opposite shift occurred in the challenging high-ranking worker due to the onset of oogenesis.

Policing of Worker Eggs

Besides attacking a potential egg-layer in the presence of established reproductives, workers may also destroy the eggs of subordinates by simply eating them. Workers often do so in ants (e.g., Kikuta and Tsuji 1999; D'Ettoire, Heinze, and Ratnieks 2004), in bees (e.g., Oldroyd et al. 2001), and in wasps (e.g., Foster and Ratnieks 2001). In *Dinoponera quadricaps*, the hydrocarbon profile of the egg surfaces resembles the cuticular profile of the egg-layers (Monnin and Peeters 1997). Thus the gamergate can recognize the rare eggs laid by the beta worker and eat them. Long-chained hydrocarbons are synthesized by the oenocytes (cells located in the hemolymph) and then transported to the cuticle and to the ovaries (Schal et al. 1998; Fan et al. 2003). This explains the relative similarity of cuticular profiles and surface profiles of eggs.

Further evidence that information on eggs can be used in the modulation of adult behavior comes from the ant *Camponotus floridanus*. Highly fertile queens show a cuticular hydrocarbon profile that is distinct from that of egg-laying workers; a similar difference is found in the hydrocarbon profiles of their respective eggs (Endler et al. 2004; Endler, Liebig, and Hölldobler 2006). Eggs laid by workers in an orphaned group are usually eaten by workers originating from a colony with a highly fertile queen. When, however, worker eggs are treated with the fractionated hydrocarbon extract of the cuticle of a highly fertile queen, a significantly higher percentage of manipulated eggs survive in comparison to worker eggs treated with the worker profile (Endler et al. 2004). This strongly suggests that the specific hydrocarbon profile of the queen protects eggs from destruction and is used as information by the workers for their decision to police an egg or not.

Evidence for Fertility Signals beyond Correlations

Behavioral and physiological manipulations are excellent tools to identify the function of a signal. Hormonal treatment of alpha workers in *Streblognathus peetersi* decreased fertility and simultaneously changed their cuticular

hydrocarbon profile. This treatment triggered two different forms of aggression in nontreated colony members: dominance interactions by high-ranking workers who attempted to become the new alpha and worker policing by low-ranking workers who immobilized the experimentally deficient alpha (Cuvillier-Hot, Lenoir, and Peeters 2004).

In *Myrmecia gulosa*, a bioassay using fractionated cuticular extracts showed that workers can differentiate between the cuticular hydrocarbon profiles of queens and infertile workers (Dietemann et al. 2003). The hydrocarbon fractions of the queen's cuticle were significantly more attractive than extracts from infertile workers. This is not simply a consequence of caste differences because the hydrocarbon profiles of queens and fertile workers are similar (Figure 10.2). This study provided the first evidence that hydrocarbon profiles are differentiated based on fertility differences, but it did not directly show the function of the signal.

The direct involvement of hydrocarbon profiles of the queen in the regulation of reproduction has been demonstrated in *Camponotus floridanus*. As explained earlier, the hydrocarbon profile of the egg surface closely matches the cuticular hydrocarbon profile of the mother (Endler, Liebig, and Hölldobler 2006). Workers are prevented from activating their ovaries by the mere presence of eggs from highly fertile queens, even though the queen is absent (Endler et al. 2004). This result suggests that workers recognize queen presence via the presence of her eggs. Since workers recognize queen eggs on the basis of their specific hydrocarbon profile, it is most likely that these hydrocarbons are also responsible for the induction of self-restraint in workers. Furthermore, since the profile of queen eggs induces self-restraint, the similar cuticular profile of the queen should have the same effect. Thus, hydrocarbon profiles on the surface of queen eggs as well as on the queen cuticle represent a queen signal that regulates reproduction in *C. floridanus*.

Smelling Fertile: Cuticular Information Is Not All or None

Unlike an eventual caste signal that would be all or none (i.e., queen or worker), cuticular hydrocarbons are correlated with degrees of fertility in either of the castes. In *Formica fusca* queens, continuous variation in egg-laying capacity is manifest as gradual changes in cuticular profiles (Hannonen et al. 2002). In *Diacamma ceylonense*, virgin egg-layers and gamergates have different levels of ovarian activity, and this is associated with distinct

hydrocarbon blends (Cuvillier-Hot et al. 2001). In queens of *Camponotus floridanus*, the hydrocarbon profile is correlated with colony size as an indicator of the queen's average fertility (Endler, Liebig, and Hölldobler 2006). Young founding queens with low egg-laying rates and colony sizes of less than 10 workers show a profile that is similar to that of workers; however, this profile changes continuously and becomes very distinct in highly fertile queens from colonies with more than 1,000 workers.

A fertility signal can convey graded information to label not only egg-layers but also high-ranking workers with intermediate reproductive potential. Rather than just ovarian activity, cuticular hydrocarbons reveal the individual hormonal state that underlies dominance and oogenesis. Low levels of juvenile hormone (JH) are characteristic of alpha workers in *S. peetersi* (Brent et al. 2006), and topical applications of a JH analog led to both decreased fertility (as measured by levels of vitellogenin in the hemolymph) and shift in cuticular profile (Cuvillier-Hot, Lenoir, and Peeters 2004). In *Polistes dominulus*, the size of the corpora allata (the principal site of JH production) was associated with variation in cuticular hydrocarbon proportions (Sledge et al. 2004). Each species investigated so far had about 50 different hydrocarbons on the cuticle, which provides sufficient variability for differences in sex, colony membership, and age to be detected, in addition to reproductive status. *Drosophila* flies have many long-chain cuticular hydrocarbons (C29–C35) within hours of emergence; as they get older, these are replaced by shorter chain hydrocarbons (C23–29) (Wicker and Jallon 1995). In the ant *Diacamma ceylonense*, workers up to 4 days in age have different hydrocarbon blends compared to older infertile workers (Cuvillier-Hot et al. 2001). In *Camponotus vagus*, *Harpegnathos saltator*, *Pogonomyrmex barbatus*, and *Myrmicaria eumenoides*, nurses and foragers can be distinguished by their cuticular profiles (Bonavita-Cougourdan, Clément, and Large 1993; Liebig et al. 2000; Greene and Gordon 2003; Kaib et al. 2000), suggesting age-related hormonal changes. Generally, a close connection among hormone activity (e.g., ecdysone), oenocyte activity, and the formation of the cuticular hydrocarbon profile is assumed (Howard and Blomquist 2005) (Figure 10.3). Application of JH III to *M. eumenoides* nurse workers resulted in a cuticular profile typical of foragers, even though they did not behave as foragers (Lengyel, Westerlund, and Kaib 2007). In *Harpegnathos saltator*, virgin queens develop 2 distinct alkadiene peaks prior to the mating flight (Liebig et al. 2000), which suggests that it is involved in sexual communication. These peaks are not present in founding queens that already lay eggs, or any of the workers.

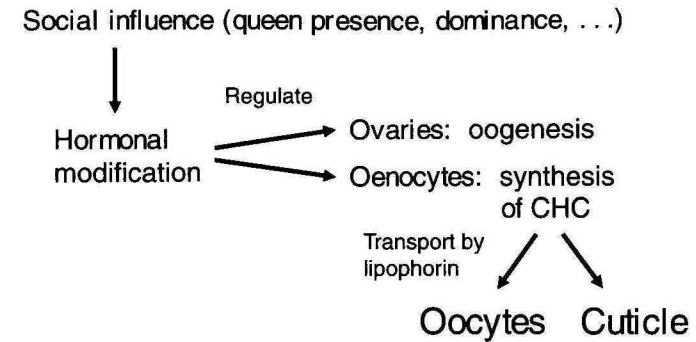


Figure 10.3. Physiological links between endocrine activity, oogenesis, and the synthesis of cuticular hydrocarbons in individual insects. Gonadotropic hormones affect both oogenesis and the activity of oenocytes. Hydrocarbons are then transported in the hemolymph to the ovaries and cuticle.

Workers laying trophic eggs (i.e., unviable yolk sacs) in *M. gulosa* have a distinct cuticular profile from that of workers producing males (Dietemann et al. 2003); that is, when a worker shifts from trophic to fertile eggs its cuticular profile changes also. This is further evidence of the influence of hormonal levels on the activity of both ovaries and oenocytes. In *S. peetersi*, cyclical egg-laying activity does not affect the profile of gamergates, which is appropriate since a temporary halt should not be perceived by colony members as a stimulus to replace their mother. In *C. floridanus*, variation in egg-laying rates among queens is high as well. Nevertheless, their profiles are very similar which indicates discontinuous egg-laying activity with a stable profile (Endler, Liebig, and Hölldobler 2006). Even though eggs may be laid in cycles, oogenesis continues and is linked to hydrocarbon synthesis.

Honest Information, Aggression, and Colony Size

Several authors (e.g., Wilson 1971; Keller and Nonacs 1993; Heinze 2004) have suggested that behavioral regulation of reproduction cannot be effective in larger colonies because a queen or dominant individual cannot interact frequently with all its nestmates. Thus, a shift from aggressive behavior to chemical signaling has been assumed to follow an increase in colony size. Yet signaling also underlies behavioral regulation since olfactory recognition is needed among contenders. Instead of invoking colony size per se, convergence or divergence of genetic interests seems more crucial to

understanding which mechanism (behavioral or pheromonal) is used. Behavioral regulation (Figure 10.4) is needed in species where all nestmates are able to mate and reproduce, and such species usually have small colonies. In contrast, in species with similarly small colonies but with dimorphic queens and workers, the former monopolize reproduction without aggression. Increasing caste dimorphism, which is always a feature of species with larger colonies, seems to eliminate the need for aggressive interactions because the interests of egg-layers and helpers converge as a result of the latter being unable to mate (Bourke 1999; Foster 2004; Dietemann, Peeters, and Hölldobler 2005, Endler, Hölldobler, and Liebig 2007).

Recognition pheromones are nonvolatile and require physical contact for transmission. Gamergates and queens separated from orphaned workers by a mesh that allowed airflow but prevented physical contact could not inhibit ovarian activation in these workers (Liebig, Peeters, and Hölldobler 1999; Liebig et al. 2000; Tsuji, Egashira, and Hölldobler 1999). In *S. peetersi*, distance of detection was measured to be 1.3mm, which is compatible with the

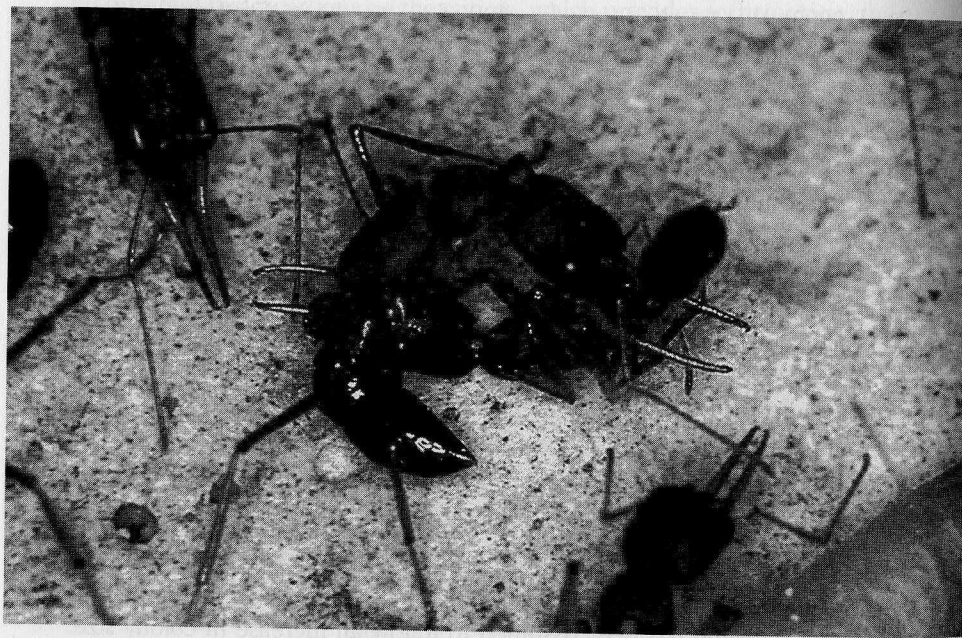


Figure 10.4. Two workers of *Harpegnathos saltator* fighting over queen succession. All workers are potentially capable of replacing a senescent queen.

involvement of long-chained hydrocarbons (Cuvillier-Hot, Renault, and Peeters 2005). In *D. quadricaps* and *S. peetersi*, the alpha worker exhibited conspicuous gaster behaviors which may help make its cuticular hydrocarbons more readily detected by competing high-ranking workers. It is commonly observed in colonies of queenless ants that high-rankers remain in close proximity to the gamergate(s); this is further evidence that colony size is unlikely to be a constraint on physical control.

In larger colonies, information about queen presence can be transmitted indirectly via hydrocarbon profiles on eggs. Workers recognize queen eggs in the ant *C. floridanus* based on their surface hydrocarbons (Endler et al. 2004). These eggs are sufficient to inhibit ovarian activity in workers and thus function as an indirect signal of queen presence. This mechanism may also prevail in polydomous colonies such as *Oecophylla*.

Conclusions and Future Directions

Fertility and Reproductive Regulation

Reproductive conflicts in insect societies differ in both proximate and ultimate characteristics: participants may belong to the same or different morphological castes and conflict resolution may manifest as either self-restraint (e.g., workers remain infertile) or aggressive behaviors. One thing in common is that all participants share the need for information about the presence of an active egg-layer. Although oogenesis also affects various exocrine secretions (e.g., Dufour's gland in *Apis*, Dor, Katzaf-Gozansky, and Hefetz 2005), cuticular hydrocarbons carry reliable information about fertility and thus play an essential role in regulating reproductive division of labor.

In species having queen and worker castes, conflicts are often restricted to male production. Senescence of the queen is followed by colony extinction in many monogynous species, so it is adaptive for workers to begin male production (e.g., Dietemann and Peeters 2000). An ageing queen is expected to have reduced fertility, which is useful information for the workers. They can either start activating their ovaries to produce males or rear exclusively sexual offspring as new workers are no longer useful.

Even in the presence of a fertile queen, some workers may behave selfishly and produce male eggs. In various species, policing workers will attack these egg-layers or destroy their eggs. In either case, this is a self-interested response to the queen's fertility signal that they can perceive either directly

or indirectly. In the absence of a fertile queen, policing workers modulate their behavior and allow some workers to produce male adults.

In *Polistes* wasps or queenless ants (i.e., all colony members have equivalent reproductive potentials), information about fertility is equally crucial. During hierarchy formation, high-ranking individuals need to recognize each other. Once an alpha begins oogenesis, there is a shift from aggression to chemical signaling. High-ranking subordinates benefit from this shift as it may stop additional aggression from lower-ranked individuals. During replacement of a senescent alpha, high-rankers need to detect a drop in fertility that makes it worthwhile for them to attempt a challenge. Policing workers can prevent a challenge while their mother is still sufficiently fertile, but will favor it after a drop in her fertility. The interests of sterile helpers converge with the gamergate's or queen's only as long as the latter is able to produce many offspring.

Testing Insect Perception of Fertility Signals

Variations in cuticular hydrocarbons give reliable information about the reproductive status of individuals in all the ants, wasps, and bees investigated thus far (Monnin 2006; Liebig and Peeters, in prep.). This information is useful for human investigators, but more evidence is needed to determine if insects use it to resolve their reproductive conflicts. Although individuals that are induced experimentally to start or stop laying eggs exhibit predictable modifications in their cuticular profiles, it is important for future research to demonstrate the pheromonal function with an artificial profile. More behavioral studies together with a better understanding of the significance of the physico-chemical properties of the different types of cuticular hydrocarbons are timely. Polar compounds on the cuticle should also be investigated (Dapporto, Dani, and Turillazzi 2008).

The cuticular hydrocarbons that co-vary with fertility are seldom the same across species. One may ask, why are all species systematically different in hydrocarbon chemistry? Is it just an epiphenomenon of hydrocarbon biosynthesis or is this diversity selected for (see Breed and Buchwald, this volume)? Although a cuticular layer of alkanes might be sufficient to prevent desiccation, it is not biosynthetically feasible to produce alkanes exclusively (R. Crewe, pers. comm.). There are many steps along the metabolic pathways involved in the synthesis of long-chained hydrocarbons (Howard and Blomquist 2005), and some of the enzymes that catalyze each step may result from differential gene expression (e.g., Ferveur and Jallon 1996).

Typically, about 50 different long-chained hydrocarbons occur in a typical ant species. Determining the specific cuticular compounds involved in the recognition of either nestmates or reproductives is a crucial step. Multivariate statistical tools can help to identify potential candidates, but do not tell us which of several molecules are behaviorally important. The most conspicuous difference may be misleading as individuals may respond to another compound or set of compounds. Systematic evaluation of the role of hydrocarbon classes would be useful. As an example, alkenes and methyl-groups are important carriers of information in nestmate discrimination in *Apis* (Dani et al. 2001; Dani et al. 2005). As a result of 3-D conformation, branched alkanes might be easier to distinguish than linear alkanes and thus more likely to be represented in a fertility signal.

Another approach to understanding the very basics of hydrocarbon recognition involves electro-antennograms (EAG). In *Pachycondyla inversa*, the major compound of the queen signal produced a significantly higher response in an EAG than other components of the profile (D'Etterre, Heinze, 2004). Although this shows that the ants have a higher sensitivity to this compound, it does not explain how the information is processed and what the natural response to the compound is. EAGs cannot replace bioassays that show the result of the processing of olfactory information (the behavioral or physiological response). They give only limited information about the potential to perceive the respective substances and may suggest receptor specialization for certain compounds. In combination with bioassays EAGs can, however, help to isolate active compounds within a complex profile.

Cues of Colonial Identity and Individual Information about Fertility

Colonial recognition based on cuticular hydrocarbons has been demonstrated in a small number of ant species (e.g., Lahav, Soroker, and Hefetz 1999; Thomas et al. 1999; Wagner et al. 2000). In *Pachycondyla goeldii*, Denis, Blatrix, and Fresneau (2006) found that the cuticular hydrocarbons responsible for the best discrimination among ovarian development classes also yielded a clear discrimination among colonies. In *Camponotus floridanus*, the hydrocarbon profile typical for the colony was clearly present in addition to the reproductive profile in queens (Endler et al. 2004), and similar results were obtained in *Diacamma ceylonense* (Cuvillier-Hot et al. 2001). Hence, the cuticular profile contains dual information about colony membership and fertility status.

According to the Gestalt model, all nestmates share a colonial odor as a

result of mixing their cuticular hydrocarbon profiles. However, as far as the various hydrocarbons that are correlated with fertility are concerned, the lack of gestalt is a reality. Furthermore, there are other intracolony differences (behavioral subcaste, age, sex) encoded in the cuticular hydrocarbon profiles. How can these intracolony differences be reconciled with the mixing of odors? Further studies are needed to verify that mutual exchange of hydrocarbons is always necessary to maintain a colony profile (a function claimed for the postpharyngeal gland, Lahav et al. 1998; Boulay et al. 2000), and the link with the genotype must be elucidated. Another possibility is that the hydrocarbons correlated with fertility are produced at a higher rate than other longer-chained hydrocarbons. This needs to be investigated empirically. Indirect support for this is the high frequency of self-grooming shown by new alphas in *S. peetersi* (Cuvillier-Hot et al. 2004). Self-grooming results in a transfer of hydrocarbons to the postpharyngeal gland via the basitarsal brush on the front legs, as shown in *Pachycondyla apicalis* (Hefetz et al. 2001). New alphas benefit from communicating their change in status as quickly as possible, and this can be achieved by removing cuticular hydrocarbons that no longer reflect their current physiological condition. It is a challenge for the future to understand how ants are able to extract multiple pieces of information from a single hydrocarbon profile. We need to identify the different active parts of the profile and separately demonstrate the ability of the ants to retrieve various kinds of information. Once this is achieved, the concept of "queen pheromone" can be usefully replaced by fertility pheromone.

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