Chemical recognition of reproductive status in social insects

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Inclusive fitness theory explains how helpers reproduce indirectly via the breeders they help. The inclusive fitness helpers get depends on their relatedness to the breeder(s), colony productivity and fertility of the breeder(s). It is therefore critical for workers to assess breeder fertility. There is strong evidence that, in wasps, bees and ants, the cuticular hydrocarbon (CHC) profiles of breeders are a signal of fertility. Chemical and behavioural evidence suggests that linear alkanes are not involved in communication, whereas methyl-branched alkanes and alkenes may constitute, or at least contribute to, the fertility signal. The correlation between CHCs and reproduction is well established, as well as the fact that CHCs are detected and that workers react accordingly. However, whether CHC profiles are honest is yet to be demonstrated. Hormonal and genetic studies, such as inactivating genes regulating the production of alkenes, are promising approaches to investigate the honesty of CHC profiles.

Introduction

Chemical communication is central to the functioning of insect societies. For instance, it is involved in the discrimination of nestmates from alien conspecifics, the collection of food, and the regulation of reproduction. Regarding this last point, Keller and Nonacs (1993) argued that breeders are unlikely to chemically coerce workers into helping. Pheromones inhibiting worker reproduction would likely hamper queen reproduction as well. Additionally, mutant workers immune to such inhibitory queen pheromones (e.g. with dysfunctional pheromone receptors) would produce males. That is, mutant workers would have direct fitness and the mutation would

invade the population, unless the colony-level costs of worker reproduction are higher than this benefit. Keller and Nonacs (1993) concluded that it is more likely that breeders advertise their high reproductive potential, and that workers help highly fertile breeders because this increases their own inclusive fitness. Indeed, kin selection theory shows that helping is favoured when $br - c > 0$, that is when the breeder is related (r) and actually benefits (b) from the help it receives. No matter how high relatedness may be and how low cost of helping may be, helping a breeder with low fertility is not beneficial. Therefore, workers are strongly selected for accurately estimating breeder's fertility. In the presence of a fertile breeder, workers should refrain from reproduc-

ing and help the breeder. But in the presence of a declining breeder, workers should raise a replacement breeder, produce males, or mate and reproduce sexually, depending on the species.

Over the last 10–15 years an increasing number of studies has investigated putative chemical signals of fertility in social Hynmenoptera, including *Polistes* wasps. In particular, cuticular hydrocarbons (CHCs) have attracted much attention. They are easily identified with GC/MS, are pheromones in solitary insects (e.g. Ferveur 2005), and are involved in communication (colony recognition) in many social insects. Here, I review the roles of CHCs in preventing desiccation and allowing colony recognition. Then I present the chemical evidence that CHCs are signals of fertility in wasps, bees and ants, followed by behavioural evidence. I proceed by reviewing experiments showing that social insects indeed perceive CHCs and can discriminate molecules differing only slightly, before briefly reviewing the hormonal factors that underlie both CHC production and fertility. I conclude by discussing whether CHCs are honest signals, analogous to those involved in sexual selection in vertebrates.

Roles of CHCs

CHCs and resistance to desiccation

CHCs are abundant in insects, where they primarily prevent desiccation (e.g. Gibbs 2002, Howard & Blomquist 2005). In social insects CHCs are mostly a mixture of linear alkanes, methyl-branched alkanes, alkenes and, in some species, less abundant chemicals such as alkadienes or methyl-branched alkenes. Alkanes and alkenes differ in their melting points, and consequently do not form a homogenous mixture (Gibbs 2002). Alkane crystals melt at higher temperatures than alkene crystals. Alkanes are thus likely to form impermeable layers of crystals on the insect cuticle, where alkenes are likely to be melted and form more permeable layers. This solid–liquid phase separation led Gibbs (2002) to suggest that the primary function of alkanes is resistance to desiccation, whereas that of alkenes is chemical communication.

This anti-desiccation function of alkanes is supported by empirical evidence. In the harvester ants (*Pogonomyrmex barbatus*) workers working outside the nest have a higher proportion of alkanes than workers remaining inside the nest (Wagner *et al.* 2001). The experimental manipulation of environmental factors shows that high temperature combined with low relative humidity (38 °C and 8% RH) increases the proportion of linear alkanes on the cuticle. Additionally, when individuals working inside the nest are forced to stay outside, in a drier environment, the proportion of alkanes on their cuticle increases (Wagner *et al.* 2001).

CHCs and colony recognition

In many social insects cuticular hydrocarbons allow colony recognition (Howard & Blomquist 2005). For instance, the topical application of (1) the hydrocarbon fraction of the postpharyngeal glands (PPG) extract, (2) the non-hydrocarbon fraction of PPG extract, or (3) the total PPG extract shows that *Cataglyphis niger* ants use hydrocarbons for colony recognition (Lahav *et al.* 1999). This is further demonstrated by the topical application of synthetic hydrocarbons, which allows manipulating an individual's odour by increasing the proportion of a focal CHC, and thus testing which compounds affect colony recognition. In honeybees adding an alkane has little effect, while adding an alkene triggers the aggression of the treated individual, which is no longer recognised as a colony member by its own nestmates (Dani *et al.* 2005). This shows that alkanes are not involved in colony recognition whereas alkenes are.

In *Polistes dominulus* and *Vespa crabro* CHCs not only differ between colonies, they also differ between related and unrelated nestmates within colonies (i.e. wasps with different mother and father following queen turnover or nest usurpation, Dani *et al.* 2004). CHCs could thus potentially provide sufficient information for nepotism to occur in colonies with several queens. However, it is unclear whether it could occur in societies with one multiple-mated queen as the chemical difference between full- and half-sisters is lower (Dani *et al.* 2004), and there

is, to date, no example of nepotism in social insects (e.g., Solís *et al*. 1998, Strassman *et al*. 2000, Gamboa 2004, Holzer *et al*. 2006).

CHCs as signal of fertility

There is good chemical evidence from wasps, bees and ants that CHCs, in addition to preventing desiccation and being involved in colony recognition, advertise an individual's reproductive activity. In all the species where it has been specifically examined, a strong correlation has been found between the reproductive status of an individual and its CHC profile, with breeders having a different blend of CHCs than non-breeders (Table 1). I review this evidence following a taxonomic approach to emphasise that the correlation between reproductive activity and CHCs is widely distributed.

CHCs and reproduction in wasps

In independent founding *Polistes* wasps, colonies are initiated by one or a few co-operating females, depending on the species. In temperate species, nests are started in spring by foundresses, that is females which had emerged the previous season and hibernated (Reeve 1991). When several foundresses initiate a nest together they aggressively compete for reproductive rights, and soon one foundress becomes dominant and more or less monopolises reproduction (functional monogyny). Subordinate foundresses may lay a few eggs but these are mostly destroyed by the alpha (e.g. Reeve 1991, Röseler 1991, Queller *et al*. 2000). Typically, the first generation of female offspring behaves as helpers, although they can alternatively mate and become foundress the next season (e.g. Starks 2001).

In *P. dominulus*, in colonies composed of only one foundress and workers, the CHC profile of the foundress differs from that of its sterile daughters (Bonavita-Cougourdan *et al*. 1991). The chemical difference is complex, involving many CHCs (Table 1). In one orphaned colony without a foundress, one worker was reproducing, and it had a foundress-like CHC profile (Bonavita-Cougourdan *et al*. 1991).

Because this work compared the foundress to her daughters, the chemical differences found could potentially stem from differences between foundresses and workers rather than differences in reproductive status: foundresses are older than workers, they hibernated, and they tend to be larger than the first generation of workers. Therefore, Sledge *et al*. (2001) studied colonies with several foundresses. At the time of nest initiation all foundresses have the same degree of ovarian development and they have identical CHC profiles. However, by the time of worker emergence the alpha foundress more or less monopolises reproduction and its CHC profile differs from that of subordinate foundresses, which now have undeveloped ovaries. Additionally, alpha and subordinate foundresses chemically differ from workers (Sledge *et al*. 2001). The removal of alpha allows beta to become dominant and to develop its ovaries, and within 10 days it develops a CHC profile typical of an alpha foundress (Sledge *et al*. 2001). Dapporto *et al*. (2005) completed this work by investigating changes in CHCs of workers laying eggs following orphaning. As expected, workers starting to lay eggs also develop an alpha-like CHC profile. Workers not laying eggs become beta-like, which was not predicted. This may result from the workers which are suppressed from reproducing by dominant egg-laying workers, readying themselves to reproduce in the event of egg-laying workers disappearing, much like subordinate foundresses are ready to inherit the nest if alpha dies.

These works conclusively show that *Polistes* breeders and non-breeders differ with respect to their CHC profile: egg-laying foundresses (alpha) differ from non-reproductive foundresses (subordinate foundresses), and in orphaned colonies egg-laying workers differ from non-reproductive workers. However, the chemicals differing between breeders and non-breeders vary between studies (Table 1). Additionally, Dapporto *et al*. (2004) found alpha profiles and beta profiles to vary between localities. They compared the CHCs of alphas and betas, after worker emergence, in three localities. Alphas from two localities had identical CHC profiles but alphas from the third locality had a different profile. Likewise, the same occurs with betas.

Table 1. Differences in the cuticular hydrocarbon profiles of egg-layers and non egg-layers.

Species	Type of individuals	CHCs over-represented in egg-layer(s)	CHCs under-represented in egg-layer(s)	References
Polistine wasps				
Polistes dominulus	alpha foundress *vs.* workers	$n\text{-}C_{31}$; $n\text{-}C_{33}$; $13\text{-},15\text{-},17\text{-}meC_{33}$; $7\text{-}meC_{33}$; $14\text{-},16\text{-}meC_{34}$; $13\text{-},17\text{-}meC_{35}$	$13\text{-},15\text{-}meC_{29}$	Bonavita-Cougourdan *et al.* 1991
Polistes dominulus	alpha *vs.* beta foundress *vs.* workers	$9\text{-}C_{29}{:}1$; $9\text{-}C_{31}{:}1$; $C_{33}{:}2$; $C_{35}{:}2$	$5\text{-}C_{29}{:}1$; $5\text{-}C_{31}{:}1$; $7,15\text{-}dimeC_{35}$; $5,15\text{-}dimeC_{29}$	Sledge *et al.* 2001
Polistes dominulus	alpha *vs.* beta foundress	$n\text{-}C_{27}{:}1$; $7\text{-}meC_{27}$ and a mix of central meC_{29} differ between alpha and beta foundresses		Dapporto *et al.* 2005
Polistes dominulus	egg-laying workers *vs.* workers	$n\text{-}C_{29}{:}1$; $2\text{-}meC_{28}$; $n\text{-}C_{33}{:}1$ and $5,y\text{-}dimeC_{33}$ differ between egg-laying and sterile workers		Dapporto *et al.* 2005
Polistes fuscatus	foundresses *vs.* workers	none	$n\text{-}C_{21}$; $n\text{-}C_{22}$; $C_{29}{:}1$; $13\text{-}meC_{31}{:}1$	Espelie *et al.* 1994
Vespine wasps				
Vespa crabro	queen *vs.* workers	$n\text{-}C_{24}$; $n\text{-}C_{27}$	$3\text{-}meC_{23}$; $C_{27}{:}1$	Butts *et al.* 1995
Apidae bees				
Bombus hypnorum	queen *vs.* workers	branched alkanes, alkadienes, terpene and terpenoid esters	saturated carboxylic acids and saturated acetates	Ayasse *et al.* 1995
Ponerine ants				
Dinoponera quadriceps	gamergate or egg-laying worker *vs.* workers	$9\text{-}C_{31}{:}1$ ($9\text{-}C_{33}{:}1$ is over-represented in both egg-layers and young workers)	none	Monnin *et al.* 1998, Peeters *et al.* 1999
Streblognatus peetersi	gamergate or egg-laying worker *vs.* workers	CHC differ between egg-layers and workers		Cuvillier-Hot *et al.* 2004a, 2004b
Diacamma ceylonense	gamergate or egg-laying worker *vs.* workers	$9\text{-},11\text{-},13\text{-}meC_{25}$; $3\text{-}meC_{25}$; $5\text{-},7\text{-},9\text{-},11\text{-},13\text{-}meC_{27}$; $3\text{-}meC_{27}$	$n\text{-}C_{29}$	Cuvillier-Hot *et al.* 2001
Diacamma sp. from Japan	gamergate *vs.* workers	"weakly volatile chemical components on the cuticle that appear to be specific for reproductive individuals"		Okai and Tsuji (as cited in Tsuji *et al.* 1999)
Harpegnathos saltator	queen and gamergate *vs.* new queens and workers	$13,23\text{-}dimeC_{37}$, specific to egg-layers; plus other non specified CHC differing between egg-layers and non egg-layers		Liebig *et al.* 2000
Pachycondyla inversa	queen *vs.* workers	$3,9\text{-}dimeC_{25}$; $5\text{-}meC_{27}$; $7\text{-}meC_{27}$; $11,15\text{-}dimeC_{27}$ and especially $3,11\text{-}dimeC_{27}$ and $3,11\text{-}dimeC_{29}$		Heinze *et al.* 2002
Platythyrea punctata	egg-laying workers *vs.* workers[#]	$9\text{-},11\text{-}meC_{23}$; $5\text{-}meC_{23}$; $2\text{-}meC_{23}$; $3\text{-}meC_{23}$; $n\text{-}C_{25}$; $7\text{-}meC_{25}$; $2\text{-}meC_{25}$	$9\text{-}C_{23}{:}1$; $8,16\text{-}C_{25}{:}2$; $2,9\text{-}C_{25}{:}2$; $9\text{-}C_{25}{:}1$; $9\text{-}pentacosyne$; $9\text{-}C_{27}{:}1$; $C_{27}{:}2$; $7\text{-}C_{27}{:}1$	Hartmann *et al.* 2005
Myrmecine ants				
Myrmecia gulosa	queen or egg-laying workers *vs.* workers	$9\text{-}C_{25}{:}1$ and $3\text{-}meC_{25}$ specific to egg-layers; plus 13 other CHC differ between egg-layers and non egg-layers		Dietemann *et al.* 2003

Myrmicine ants				
Temnothorax acervorum	queen *vs.* workers	queens differ from workers in the proportion of most CHC		Tentschert *et al.* 2002
Temnothorax gredleri	queen *vs.* workers	queens differ from workers in the proportion of most CHC		Tentschert *et al.* 2002
Formicine ants				
Cataglyphis iberica	queen *vs.* workers	n-C_{27}; n-C_{28}; n-C_{29}; $dimeC_{29}$	11-,13-meC_{27}	Dahbi and Lenoir 1998
Camponotus floridanus	queen *vs.* workers	queen differs from workers in the proportions of alkanes and branched alkanes		Endler *et al.* 2004
Camponotus vagus	queen *vs.* workers	queen differs from workers in the proportions of alkanes and branched alkanes		Bonavita-Cougourdan & Clément 1994
Formica fusca	Highly fertile queen *vs.* less fertile queen	3-meC_{23}; 5-meC_{25}; $9,13$-$dimeC_{25}$; $5,13$-$dimeC_{25}$ and $3,y$-$dimeC_{27}$		Hannonen *et al.* 2002
Dolichoderine ants				
Linepithema humile	queen *vs.* non laying queens and workers	queens differ from non laying queens and workers in the proportion of most CHC		de Biseau *et al.* 2004

workers reproduce by thelytokous parthenogenesis

This apparently goes against the hypothesis that CHCs are an honest signal, because one does not expect a signal presumably linked to physiology to vary significantly between relatively close localities. For instance, if the CHC profile depends on reproductive hormone titters, alphas should bear the same characteristic CHCs irrespective of locality. However, Dapporto *et al.* (2004) performed a Discriminant Analysis of the CHC profiles using alphas from each locality and betas from each locality as six *a priori* groups; that is, they looked for differences between ranks OR localities. Separating individuals according to locality may have hampered finding consistent differences between ranks only. Indeed, feeding on different prey can alter the CHC profile (Liang & Silverman 2000, Buczkowski *et al.* 2005), and such differences may be superimposed upon reproduction differences. Ignoring locally-induced differences in order to highlight the differences solely due to reproduction could be achieved by using alphas from all localities, betas from all localities and workers from all localities as three *a priori* groups, respectively.

CHC differences between breeders and workers have also been found in *Polistes fuscatus* (Espelie *et al.* 1994) and in the hornet *Vespa crabro* (Butts *et al.* 1995). Although it is clear that breeders chemically differ from sterile individuals in these three species, no pattern emerges. Breeders tend to be characterised by some alkenes and methyl-branched alkanes in *P. dominulus*, whereas this is not true in *P. fuscatus* and *V. crabro* (Table 1). Additionally, it remains unclear whether this difference is an honest signal of fertility.

CHCs and reproduction in bees

Chemical differences between breeders and sterile helpers also occur in the bumblebee *Bombus hypnorum* (Ayasse *et al.* 1995). The CHC profile of the queen differs from that of workers, and the CHC profiles of workers vary with age and ovarian activity, with dominant egg-laying workers having a CHC profile similar to that of queens. Additionally, the amount of CHCs is more closely correlated to ovarian activity than to body size (Ayasse *et al.* 1995).

Similarly, CHCs may advertise fertility in *Bombus terrestris*. As in most social Hymenoptera workers reproduce when orphaned, but they refrain from doing so when queen's cuticular extract is deposited onto a callow queen (Bloch & Hefetz 1999a). Furthermore, in orphaned colonies dominant egg-laying workers inhibit other workers' reproduction (Bloch & Hefetz 1999b).

CHCs and reproduction in ants

Most evidence that CHCs advertise reproductive status come from ants. These ants are a diverse set of species, both taxonomically and in regard to the reproductive potential of workers. The ant species studied include some queenless ants which are comparable to *Polistes* wasps in having no morphological queen, but workers capable of mating and reproducing sexually. Typically, only one or a few workers do mate. Mated egg-laying workers are called gamergates to highlight that they function as queens but are morphologically workers. Other ant species studied have morphologically specialised queens and workers which cannot mate, and thus only produce males in orphaned colonies. In Argentine ants workers lack ovaries altogether and cannot reproduce at all. Finally, in *Platythyrea punctata* workers cannot mate but nevertheless produce other workers, by thelytokous parthenogenesis. This diverse set of species strongly suggests that CHCs are a signal of fertility in all ants.

In the monogynous queenless ant *Dinoponera quadriceps*, the CHC profile of the gamergate differs from that of the other workers, with the gamergate having higher proportions of $9\text{-}C_{31}{:}1$ and $9\text{-}C_{33}{:}1$ (Monnin *et al.* 1998). Young workers also have a high proportion of $9\text{-}C_{33}{:}1$, so that only $9\text{-}C_{31}{:}1$ is characteristic of the gamergate. When the gamergate is removed, beta, or occasionally gamma or delta, soon behaves very aggressively and establishes itself as the new alpha. It then activates its ovaries and produces male-destined eggs (Monnin & Peeters 1999). Aggressiveness decreases back to normal in a few days and the new alpha's CHCs become gamergate-like (i.e. rich in $9\text{-}C_{31}{:}1$) in ca. six weeks (Peeters *et al.* 1999). This new alpha may mate and become a gamergate (Monnin & Peeters 1998). However, the mating status does not affect the CHC profile, and virgin alphas do not differ chemically from gamergates (Monnin *et al.* 1998). That is, CHCs advertise fertility but not mating status.

Similarly, in *Streblognathus peetersi* the CHCs correlate with fertility. The gamergate, high-ranking workers and low-ranking workers each have characteristic CHC profiles (Cuvillier-Hot *et al.* 2004b). As occurs in *D. quadriceps*, when the gamergate is removed, beta, gamma or delta behaves very aggressively during a few days to firmly establish itself as the new alpha. It then starts to lay eggs and, in parallel, its CHC profile gradually becomes gamergate-like (Cuvillier-Hot *et al.* 2004b).

Diacamma ceylonense is yet another queenless ant where the CHC profile varies with age and fertility (Cuvillier-Hot *et al.* 2001). In addition there is, again, a gradual shift from an initially aggressive monopolisation of reproduction by a virgin worker (alpha) towards a peaceful maintenance of the established breeding structure. Simultaneously, the CHCs of alpha change from callow-like towards gamergate-like (Cuvillier-Hot *et al.* 2002).

A strong correlation between CHCs and reproductive activity is also found in species having both gamergates and queens (i.e. females morphologically specialised for reproduction). In *Harpegnathos saltator*, a single queen independently starts the colony, and she is replaced by several gamergates when she dies (Peeters & Hölldobler 1995). The CHC profiles of both types of breeders correlate with oogenesis. Gamergates and queens have similar CHC profiles, and they differ from workers and from young queens which are awaiting the time to leave the colony to start their own (i.e. gynes). 13,23-dimethylheptatriacontane ($13,23\text{-diMeC}_{37}$) is highly characteristic of gamergates and queens, which additionally differ from sterile individuals by having longer-chained CHCs (Liebig *et al.* 2000). When the gamergates are removed some young workers become aggressive, lay eggs and experience a modification of their CHC profile from worker-like to breeder-like. Some of the workers who initially become dominants and acquire a breeder-like CHC profile subsequently lose their social rank and experience a reversal to a worker-like CHC profile (Liebig *et al.* 2000).

Only relatively few ant species have gamer-gates (Peeters & Ito 2001), meaning the strong correlation between CHC profiles and reproductive activity found in these species may not be representative of ants in general. However, this correlation has been confirmed in other ants. For instance, in *Pachycondyla inversa* CHCs allow the queen to be distinguished from workers, and in orphaned colonies it allows workers with various levels of reproductive activity to be distinguished (ovaries undeveloped, slightly developed, well developed and fully developed, Heinze *et al*. 2002). Several CHCs differ between reproductive and non-reproductive individuals, but one is particularly important: 3,11-dimethylheptacosane (3,11-diMeC$_{27}$) represents around 5% of the CHCs in non-reproductive workers, versus 35% in egg-laying orphaned workers, and 50% in the queen. Chemical analysis of workers before and after queen removal shows that their CHCs change from worker-like to queen-like, with a 4-fold increase in 3,11-diMeC$_{27}$ and 3,11-diMeC$_{29}$ (Heinze *et al*. 2002).

All the above species belong to the archaic subfamily Ponerinae. However, species where CHCs correlate with reproduction have been found in most major ant subfamilies, namely Myrmeciinae, Myrmicinae, Formicinae and Dolichoderinae (Table 1). In the archaic ant *Myrmecia gulosa* (Myrmeciinae), the CHC profile of queens differ from that of young queens (gynes) and workers, and in orphaned colonies egg-laying workers also differ from gynes and workers (Dietemann *et al*. 2003). 9-pentacosene (9-C$_{25}$:1) and 3-methylpentacosane (3-MeC$_{25}$) are almost exclusively found in breeders (queens or egg-laying workers), which also differ from non-breeders (gynes or workers) by the proportions of 13 other CHCs. Dietemann *et al*. (2003) presented purified cuticular extracts of queens or workers to workers. The CHC fraction of queen extracts elicited higher interest in workers than the non-CHC fraction of queen extracts or than worker extracts, which shows that workers perceive CHCs and discriminate queen and worker CHC profiles. However, the total queen extract (CHC fraction + non-CHC fraction) elicited even higher interest in workers, which shows that chemicals other than CHCs are also involved (Dietemann *et al*. 2003).

In *Temnothorax acervorum* and *T. gredleri* (Myrmicinae) queens and workers differ in the proportions of most CHCs (Tentschert *et al*. 2002). The authors analysed the CHC profiles with the principal component analysis which showed that queens from all colonies tend to cluster together, especially in *T. gredleri*. Presumably, this queen/worker difference would be even greater if analysed with a discriminant analysis. Unlike the principal component analysis, it emphasises the differences that are consistent in *a priori* groups (e.g. queen *vs*. worker), thereby excluding differences that may relate to colony odour and diet rather than reproductive activity.

In *Cataglyphis iberica* (Formicinae), the queen and sterile workers have different CHC profiles, and queens have CHC profiles more similar to one another than to their respective workers (Dahbi & Lenoir 1998). This supports the idea that CHCs advertise fertility. However, in the closely related *Cataglyphis niger* Lahav *et al*. (2001) found that "the queen profile was indiscriminable from that of the workers and was often at the centre of the group". This is because the authors were not studying reproduction, but were studying colony recognition. Therefore, they performed a discriminant analysis using colony membership as an *a priori* grouping; that is, they tested whether some CHCs differed between colonies irrespective of castes, so that queens and workers from each colony are grouped together. Using castes as *a priori* groups may have separated queens from workers, as occurs in *C. iberica*.

In *Camponotus floridanus* (Formicinae), the queen differs from workers in its CHC profile (Endler *et al*. 2004). Similarly, in *Camponotus vagus* the queen and workers differ chemically during spring and summer, when the queen lays many eggs, but do not differ at the end of winter when the queen has not yet started to lay eggs (Bonavita-Cougourdan & Clément 1994 as cited in de Biseau *et al*. 2004). This shows that CHCs vary with egg-laying, which supports the hypothesis that they honestly advertise fertility.

Using experimental *Formica fusca* (Formicinae) colonies with two queens, Hannonen *et al*. (2002) showed that queens differing in reproductive output also differ in CHC profiles and in the degree of attention they get from workers. This

suggests that workers perceive CHC differences between queens and discriminate queens accordingly.

In the highly polygynous Argentine ant *Linepithema humile* (Dolichoderinae), reproductive queens, young queens (gynes) and workers differ in their CHC profiles, with queens having shorter CHCs. The CHC profile does not advertise mating status since mated and virgin queens are chemically identical when they have similar egg-laying rates (de Biseau *et al.* 2004). In *L. humile* workers lack ovaries, so there is no need for regulating worker reproduction. However, queens may benefit from signalling their fertility if they compete for worker attention and colony resources, and because most queens are executed yearly in *L. humile* (Keller *et al.* 1989).

The last evidence for the implication of CHCs as fertility signal comes from the parthenogenetic ant *Platythyrea punctata* (Ponerinae). All workers can potentially reproduce by thelytokous parthenogenesis but only one worker usually does, and she chemically differs from sterile workers (Hartmann *et al.* 2005). When groups of workers are isolated 27 days from the egg-layer some workers start to lay eggs, yet they still have a CHC profile typical of sterile workers. However, when groups are isolated for 47 days egg-laying workers acquire the CHC profile typical of reproductives (Hartmann *et al.* 2005). This temporal discrepancy between changes in fertility and in CHCs show that fertility and CHCs are not directly linked, but presumably regulated by common underlying factors.

The examples from aforementioned wasps, bees and ants establish beyond any doubt that CHCs correlate with fertility in many social Hymenoptera, with or without a morphological queen caste. CHCs may thus constitute, or at least contribute to, a signal of fertility.

CHCs on the surface of eggs

In addition to advertising the fertility of their bearers, CHCs can also be found on eggs. Eggs chemically marked this way can be vectors propagating the fertility signal throughout the nest (Endler *et al.* 2004), and/or they may be protected from selective egg eating.

In *Polistes dominulus* selective oophagy occurs at the founding stage, with alpha eating the subordinate foundresses' eggs (Gervet 1964). Oophagy also occurs after worker emergence, with alpha eating the few worker-laid eggs and workers eating each other's eggs (Liebig *et al.* 2005). Selective destruction of worker-laid eggs requires breeder- and worker-laid eggs to differ chemically. This seems to be the case in several ant species. In *Dinoponera quadriceps*, gamergate-laid eggs are rich in $9\text{-}C_{31}{:}1$ and are not destroyed, whereas worker-laid eggs have less $9\text{-}C_{31}{:}1$ and are eaten by the gamergate. Eggs laid by foreign gamergates, also rich in $9\text{-}C_{31}{:}1$, are less often destroyed than eggs laid by nestmate workers (Monnin & Peeters 1997). In *Pachycondyla inversa*, worker-laid eggs, produced in orphaned colony fragments, are eaten by workers when introduced in the queen-right fragment. Worker- and queen-laid eggs differ chemically, with the latter having more $3,11\text{-dimeC}_{27}$, the CHC predominant on the cuticles of egg layers (D'Ettorre *et al.* 2004a). Finally, in *Camponotus floridanus* queen-laid eggs are not destroyed and they inhibit worker reproduction. In contrast, worker-laid eggs are destroyed, but less so if they are covered with queen CHC extracts (Endler *et al.* 2004). The destruction of worker-laid eggs, either by other workers or by the breeder (policing, e.g. Ratnieks 1988, Monnin & Ratnieks 2001), is common in social Hymenoptera (e.g. Vespine wasps, reviewed in Foster & Ratnieks [2001a]; *Vespula vulgaris*, Foster & Ratnieks [2001b]; *Dolichovespula sylvestris*, Wenseleers *et al.* [2005]; queenless ants, reviewed in Monnin & Ratnieks [2001]; *Diacamma* sp., Kikuta & Tsuji [1999]; *Diacamma ceylonense*, Cuvillier-Hot *et al.* [2001]).

Myrmecia gulosa is one species where worker-laid eggs are not destroyed. In the presence of the queen workers produce trophic eggs only. But workers experimentally deprived from contact with the queen start to produce viable male eggs. These are chemically very similar to queen-laid eggs (2/3 are within the 95% confidence interval of queen-laid eggs, Dietemann *et al.* 2005). As egg-laying workers are themselves chemically intermediate between sterile workers and queens it is possible that the same CHCs are involved. Worker-laid eggs are not policed,

either because they are chemically too similar to queen-laid eggs, or because worker-laid eggs seldom occur in nature, as would-be reproductive workers are detected and policed before they can reproduce (Dietemann *et al.* 2005).

In *Polistes dominulus*, brood abundance is another cue used to assess foundress fertility: removing half of the brood every other day increases egg-laying by workers in comparison with control colonies (Liebig *et al.* 2005). How workers assess brood abundance is still unknown. They may detect a decrease in the number of brood items, either directly when they check the cells, or indirectly through a lower food demand or a lower production of brood pheromone.

Additional evidence for the implication of CHCs as signal of fertility

In most social Hymenoptera workers have retained ovaries and can produce haploid male-destined eggs when orphaned. This feature has proven useful to design bioassays which examine whether workers perceive the presence of their queen or not. Liebig *et al.* (1999) split colonies of the ant *Harpegnathos saltator* in two parts. One part contained all the gamergates and half of the workers, and it was separated with a mesh from the other part which contained workers only. Workers isolated from gamergates this way start to reproduce. This strongly suggests that they do not perceive the presence of gamergates on the other side of the mesh, presumably because gamergates advertise their presence and fertility by non-volatile pheromones. Workers isolated from the gamergates started to reproduce and develop a CHC profile intermediate between gamergates and workers. When reintroduced with the gamergates after 40 days they are recognised and aggressed by sterile workers, presumably on the basis of their intermediate CHC profile. Workers isolated for 90 days are chemically indistinguishable from gamergates, presumably because they have had more time to become fully fertile, and they are not policed (Liebig 1998).

A similar experiment was carried out by Tsuji *et al.* (1999) in the queenless ant *Diacamma* sp. from Japan. They used plastic strings glued on the thorax of workers, perpendicularly to the longitudinal axis of the body, to prevent the passage of these workers through narrow passages separating the nests into two parts, thereby restraining the movement of the treated ants to half of the nest only. The single gamergate and 1/3 of the workers were treated and restrained to one side of the nest (control workers), a second 1/3 of the workers were equally treated and restrained to the opposite side of the nest (orphaned workers), and the remaining 1/3 of the workers were not treated and thus free to move to either side of the nest (free workers). As occurred in *H. saltator*, orphaned workers soon started to reproduce, which suggests that the gamergate advertises its presence and fertility by non-volatile chemicals. It also shows that workers free to move around the nest do not propagate gamergate-produced chemicals throughout the nest, as messenger honeybees do.

In *Streblognathus peetersi*, when the gamergate is removed a worker rapidly becomes dominant in the colony, and its CHC profile accordingly changes within a few days (Cuvillier-Hot *et al.* 2004b). Reintroduction of the gamergate in the colony after 48 hours typically results in the gamergate aggressing this pretender and in workers immobilising it (Cuvillier-Hot *et al.* 2005, cf. Monnin *et al.* 2002). This suggests that 48 hours is sufficient for the pretender to start to change chemically. In contrast, when the separation lasts less than 48 hours the pretender has not sufficiently differentiated to trigger aggression by the gamergate, which usually regain its status without expressed conflict (Cuvillier-Hot *et al.* 2005).

Gnamptogenys menadensis colonies are headed by a queen or by several gamergates. Gamergate colonies were split to create a group of workers with gamergates and a group of orphaned workers, where some workers soon start to lay eggs. When both groups are reunited workers immobilise the newly differentiated egg-layers, which have an ovarian development intermediate between sterile workers and gamergates (Gobin *et al.* 1999). Mesh experiments similar to that conducted in *H. saltator* (Liebig *et al.* 1999) showed that the signal is non-volatile, suggesting that it could be CHCs (Gobin *et al.* 1999).

In *Myrmecia gulosa*, orphaned workers introduced into a foreign colony are significantly more attacked if they are egg-layers than if they are not, suggesting that the characteristic CHC profile of egg-laying workers is responsible for this discrimination (Dietemann *et al.* 2005). Additionally, Dietemann *et al.* (2005) used copper wires tied around the thorax to restrain the movement of individual ants between nest chambers separated by a small opening (cf. Tsuji *et al.* 1999). Control workers were restrained in one chamber together with the queen, orphaned workers were restrained in another chamber away from the queen, and free workers were unrestrained and therefore free to move around the nest. Dissections showed that 25% of orphaned workers start to lay eggs, whereas control and free workers do not (Dietemann *et al.* 2005). This shows that orphaned workers do not detect the presence of the queen, presumably because it advertises its presence and fertility by non-volatile pheromones. Orphaned workers with a CHC profile intermediate between reproductive workers and sterile workers are policed (immobilised), mostly by free workers (Dietemann *et al.* 2005).

Finally, in the polygynous ant *Temnothorax* sp. A, the attention a queen gets from workers depends on its fertility. Additionally, the alpha queen preferentially attacks the most-fertile subordinate queen, because "*chemical cues linked to the ovarian development of queens enable other queens and workers to discriminate among queens*" (Ortius & Heinze 1999).

Perception of CHCs

There is much chemical and behavioural evidence that CHCs constitute, or at least significantly contribute to, a signal of fertility. This is also supported by recent works demonstrating that social insects perceive CHCs and can discriminate between compounds that differ only slightly.

In *Polistes dominulus,* the topical application of synthetic linear alkanes on live wasps modifies their CHC profiles (enriched in alkanes) but this has no effect on their acceptance by fellow colony members (Dani *et al.* 2001). In contrast, wasps whose CHC profiles are altered with methyl-branched alkanes or with alkenes are attacked upon returning to their nest. This shows that wasps detect the alteration of the CHC profile, and that linear alkanes have no communication value whereas methyl-branched alkanes and alkenes are good candidate molecules for fertility signals (Dani *et al.* 2001). This is in agreement with Gibbs' (2002) suggestion that the chemical properties of alkanes and alkenes make the former well-suited to prevent desiccation and the latter likely to be involved in chemical communication.

That alkanes are poorly perceived is further supported by work on the honeybee, where the Proboscis Extension Response (PER) allows actually asking individual bees whether they perceive a chemical compounds. Using Pavlovian conditioning, honeybee workers learn to discriminate between hydrocarbons that are rewarded with a sucrose solution and hydrocarbons that are not rewarded. Honeybees failed to discriminate three linear alkanes (C_{27}, C_{29} and C_{31}) and two alkenes (9-C_{31}:1 and 10-C_{33}:1), and succeeded in discriminating five alkenes (9-C_{25}:1, 9-C_{27}:1, 8-C_{29}:1, 9-C_{29}:1 and 10-C_{31}:1, Châline *et al.* 2005). Why some alkenes are discriminated, whereas others with very similar structure are not, remains unclear (e.g. 10-C_{31}:1 versus 9-C_{31}:1 and 10-C_{33}:1), yet the trend is that alkanes are not discriminated whereas some (most?) alkenes are. Also using the PER, Fröhlich *et al.* (2001) showed that honeybees discriminate extracts of cuticular ester from drones, food storers, foragers, and queen attenders. They also discriminate extracts of cuticular polar compounds, but they fail to discriminate extracts of cuticular hydrocarbons. Châline *et al.* (2005) argued that this is because Fröhlich *et al.* (2001) tested cuticular extracts of bees from the same colony, which the tested bee would not discriminate as she recognised them as nestmates. Also in honeybees, Dani *et al.* (2005) added a single CHC on bees returning to the hive and monitored the effect of this modification of their colony odour on the behaviour of other workers. The adjunction of one alkane induced no behavioural change in guard bees, suggesting that alkanes are not involved in colony recognition. In contrast, adding one alkene triggered aggression of the treated bee, which proves that

alkenes contribute to the colony label and are detected.

Electroantennography (EAG) is another way to investigate CHC perception. In the ant *Pachycondyla inversa* 3,11-dimethylheptacosane (3,11-diMeC$_{27}$) correlates with reproductive activity (Heinze *et al.* 2002), and gas chromatography-electroantennography (GC-EAG) reveals that workers detect this methyl-branched alkane (D'Ettorre *et al.* 2004b).

These works show that linear alkanes are not detected and/or not discriminated, and are therefore probably not involved in communication. This is presumably because linear alkanes are relatively featureless molecules and crystallise at ambient temperature (Gibbs 2002). The main function of linear alkanes may be resistance against desiccation. In contrast, methyl-branched alkanes and alkenes are well discriminated and are likely to contribute to the fertility signal.

CHCs, vitellogenin, and hormones

Overall, CHCs correlate well with reproductive activity. Yet, there are some discrepancies. Several examples show that when an individual's fertility changes, either increasing or decreasing, there can be a delay in the corresponding change in its CHC profile. This does not mean that CHCs are not honest signals of fertility, because it is possible that fertility and CHC profiles react at different speeds to common underlying mechanisms. The production of reproductive hormones is an example of an underlying mechanism.

In *Polistes dominulus* the relationship between the size of *corpora allata* (and thus juvenile hormone titre, JH), ovarian activity (and thus ecdysteroid titre), and the CHC profile is complex and varies depending on the social environment. At the founding stage the dominant foundress has larger and more active *corpora allata* than subordinate foundresses (Röseler *et al.* 1980), and injection of JH and/or ecdysone increases the probability that the treated foundress becomes alpha and monopolises reproduction (Röseler *et al.* 1984). Ovariectomised alpha foundresses maintained their social dominance but failed to suppress reproduction by subordinate foundresses (Röseler & Röseler 1989), which shows that

dominance is insufficient to inhibit reproduction and that ovarian activity is involved in suppressing reproduction in nestmates. One possibility is that ecdysteroids produced by developed ovaries are involved in the production of the CHC profiles characteristic of foundresses, so that ovariectomy and its associated decline in ecdysteroids results in changes in CHCs.

Sledge *et al.* (2004) found that solitary foundresses have an ovarian activity equal to that of alphas from co-operative nest foundations, yet they have smaller *corpora allata* and a different CHC profile. This is apparently in disagreement with the hypothesis that CHCs advertise fertility. However, the authors performed a discriminate analysis using solitary foundresses, alpha of pleometrotic associations, and subordinates of pleometrotic associations as *a priori* groups, that is they looked for differences between solitary foundresses and alphas rather than similarities. Using different *a priori* groups (e.g. solitary foundresses + alphas *vs.* betas *vs.* gammas before worker emergence; solitary foundresses + alphas *vs.* subordinates *vs.* workers after worker emergence) would have tested whether solitary and social breeders show similarities in their CHC profiles.

In *Polistes canadensis* JH titre correlates well with ovarian development. Workers have low JH titre and undeveloped ovaries, whereas foundresses have higher JH titre and more developed ovaries (Giray *et al.* 2005). In foundresses, variation in JH titre is well correlated with variation in ovarian activity. In contrast, in workers, JH does not correlate with ovarian development, which could stem from the fact that JH and ovarian development vary little in workers. The topical application of methoprene, a JH analog, has no effect on the reproductive activity of workers, which suggests that the effects of JH vary depending on the physiology of the individual (Giray *et al.* 2005).

In *Bombus terrestris* egg-laying workers have significantly higher JH titres, *in vitro* JH biosynthesis rates and ovarian development than nonlaying workers, both in queenright colonies at the competition stage and in experimental queenless groups. JH titres correlate with ovarian development, but JH biosynthesis rates are neither correlated with JH titres nor with ovarian develop-

ment (Bloch *et al.* 2000b). In queens ecdysteroid titres are positively correlated with reproductive activity, whereas in workers they are correlated with reproduction in orphaned colonies but not in queenright colonies, where some egg-laying workers have low titres (Bloch *et al.* 2000a).

In *Streblognathus peetersi*, vitellogenin levels differ between the gamergate and sterile workers, with high rankers having intermediate levels (Cuvillier-Hot *et al.* 2004b). Topical application of pyriproxyfen (PPN, a JH analogue) on the gamergate does not affect her aggressiveness but decreases her fertility and triggers her immobilisation by low rankers and, eventually, her replacement by a high ranker (usually beta or gamma). This is associated with changes in the CHCs of the immobilised gamergate and of the new alpha, the former becoming more worker-like while the latter becomes more gamergate-like (Cuvillier-Hot *et al.* 2004a). This shows that both CHC profiles and fertility are linked to hormone levels (Cuvillier-Hot *et al.* 2004a).

These works show that the relationships between fertility, hormonal levels and CHC profiles are indeed complex. The effect of hormones can vary according to the social condition or the physiology of individuals, and there may be delays between changes in hormonal titres, activation/deactivation of reproductive organs and modifications of behaviour. Despite this complexity studies of the hormonal regulation of both ovarian activity and synthesis of CHCs are necessary to determine how they are connected and demonstrate whether CHCs are an honest signal of fertility.

Discussion

In social insects, workers refrain from reproducing because they get a higher inclusive fitness by helping the queen, provided that it is related and fertile. Workers would not benefit from helping a non-fertile queen, so that the queen has to advertise its fertility to obtain their help, and this signal needs to be honest. There is much evidence that cuticular hydrocarbons (methyl-branched alkanes and alkenes) form or contribute to such a fertility signal. This evidence is chemical, behavioural and hormonal, and comes

from ca. 20 species of wasps, bees and ants, with or without morphological queens.

The CHC profile does not simply indicate that an individual reproduces or not, it advertises its level of reproductive activity. Indeed, CHCs vary gradually from the profile of a sterile individual to that of a partially fertile individual, and up to the profile of a fully fertile individual. When a sterile individual becomes fertile its CHCs change accordingly and, conversely, when a fertile individual becomes sterile its CHCs change in the opposite direction. However, the correlation between CHCs and fertility is not always perfect, because they do not vary at the same rate. This does not mean that the correlation is weak, or that CHCs are not an honest reflection of fertility. Both factors may be governed by the same underlying mechanisms but may react at different speeds. Apart from the transitory moments when fertility changes rapidly, either increasing or declining, CHC profiles are good indicators of fertility.

Because CHCs have several functions, such as preventing desiccation, advertising sex, colony membership and reproductive status, it has not yet been possible to identity which molecules are part of the fertility signal. Fertile and infertile individuals clearly differ in their CHC profiles, but no pattern emerges. There is a tendency for egg-layers to have specific chemicals that non egg-layers lack, rather than the opposite pattern, and in particular for egg-layers to have more methyl-alkanes. However, in some species egg-layers have more alkenes or alkadienes (e.g. *Bombus hypnorum*, *Dinoponera quadriceps*) but in other species it is non egg-layers who have more (e.g. *Polistes fuscatus*, *Platythyrea punctata*, Table 1). Methyl-alkanes and alkenes are good candidate molecules to signal fertility, whereas linear alkanes are likely to prevent desiccation and play no role in communication (Gibbs 2002).

The correlation between reproduction and CHC profiles is now well documented, as well as the fact that CHC profiles are detected and that individuals react accordingly (e.g. by not reproducing or by policing egg-laying workers). That is, there is much support for the hypothesis that CHC profiles function as signals. However, whether this signal is honest remains to be dem-

onstrated. Zahavi's handicap principle proposes that signals can be honest if their production is constrained or costly, so that the signal cannot be counterfeited or that it is not profitable to do so, respectively (e.g. Alcock 1993, Krebs & Davies 1997). CHCs could be an honest signal if breeders produce a different set of CHCs than non-breeders, and if non-breeders cannot mimic a breeder's blend, either because of physiological impossibility or because of associated costs (e.g. workers often aggress workers with a CHC profile intermediate between sterile workers and breeders). One hypothesis is that only breeders can produce the right CHC mixture because their physiology differs from that of workers since they have active ovaries and characteristic hormone titre. More recently, studies have also shown that insects can detect and discriminate between slightly different CHCs. These findings support the hypothesis that CHCs are honest signals of fertility. However, the available evidence is, to date, simply correlative.

Some of the molecules that form the CHC profiles may be active (i.e. be part of the fertility signal) whereas others may not. More experimental studies are thus necessary to identify which molecules are active, for instance by modifying the CHC profile of live individuals by the adjunction of a single CHC (Dani *et al*. 2001, 2005), or by conditioning individuals (Châline *et al*. 2005). Electroantennography would restrain the number of chemicals to be behaviourally tested by identifying which compounds are detected by the antenna and thus have the potential to be part of the fertility signal (D'Ettorre *et al*. 2004b). It is possible that active molecules contribute to the fertility signal only, but it is also possible that some molecules simultaneously contribute to other signals (e.g. be part of the colony odour label, or the sex signal), so that the various signals are in part super-imposed.

New developments may come from hormonal and genetic studies. More research is necessary on the hormonal regulation of both CHC production and ovary activity. Finding common underlying mechanisms would add support to the hypothesis that CHCs are an honest signal of fertility (Cuvillier-Hot *et al*. 2004a, Giray *et al*. 2005). Also, the manipulation of genes regulating CHC production opens exciting possibili-

ties. In *Drosophila melanogaster* the mutation of one gene decreases the production of alkenes and alkadienes and increases the production of alkanes (Marcillac *et al*. 2005). Transposing this technique to social insects could yield mutant females producing low amounts of alkenes, which are often important components of the CHC profile characteristic of breeders (Table 1). This would allow testing the honesty of the CHC profiles of breeders. Mutant queens producing small amounts of alkene should fail to enlist the cooperation of workers, so that workers should reproduce despite her presence. Also, egg-laying mutant workers producing little alkenes should not be detected and policed by nestmates.

Acknowledgements

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