

Cuticular hydrocarbons correlate with fertility, not dominance, in a paper wasp, *Polistes dominulus*

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Abstract Cuticular hydrocarbons (CHCs) are information-rich signals in social insects that coordinate behaviors within nests. However, in some taxa, the precise information conveyed by CHCs is poorly understood. In particular, there is a debate over whether CHCs convey information about their bearer's dominance or fertility. Distinguishing between dominance and fertility signaling is difficult because fertility and rank are frequently correlated within social insect colonies. This study disentangles those relationships by examining CHCs of *Polistes dominulus* paper wasps during the early nest-founding stage before dominance and fertility become correlated. First, we confirm that dominance and fertility are not associated in early spring foundresses. Then we show that CHCs are more strongly associated with fertility than dominance. There was no relationship between cuticular hydrocarbon profiles and a wasp's ability to dominate rivals through aggression, suggesting that CHCs are unlikely to provide information about dominance. However, there was a significant correlation between ovarian development and the CHC profile, suggesting that CHCs could convey valuable information about their bearer's fertility. Furthermore, our data provide a potential mechanism for chemical signaling of fecundity, as there is a relationship between

endogenous juvenile hormone titer (a gonadotropin), degree of ovarian development, and the CHC profile. Hormonal regulation of CHC profile expression offers a physiological mechanism to coordinate behavior, physical state, and signal expression.

Keywords Queen pheromone · Worker policing · Agonistic signaling · Ovarian development · Fertility · Dominance

Introduction

Social insect colonies are a fascinating mixture of cooperation and conflict. As a result, there is interest in the behavioral mechanisms that coordinate colony dynamics in the face of strong reproductive competition. Communication is one important way that social insects mediate conflict and promote cooperation. Signals are used to coordinate a variety of behaviors, from nestmate recognition and caste assessment to foraging and colony defense (Hölldobler and Carlin 1987).

Much social insect communication is chemical, and the major source of chemical information in social insect colonies is the cuticular hydrocarbon (CHC) profile. CHCs form a waxy covering on the outer layer of all insects that reduces water loss and prevents desiccation. The CHC profile is a complex, highly variable mixture of compounds that has the potential to convey valuable information (Hölldobler and Carlin 1987; Hölldobler 1999). As a result, there has been extensive research on the information content of CHCs. In both social and non-social species, CHC profiles convey information about their bearer's status and sex, but in the social insects, CHCs are best known for their role in nestmate recognition (Lorenzi et al. 1997; Gamboa 2004). In ants, cuticular hydrocarbons reliably

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provide information regarding fertility, and help mediate aggression within a nest by identifying reproductive cheaters (Peeters and Liebig 2009; Smith et al. 2009).

Although there has been extensive work on CHCs in ants, less is known about the information conveyed by CHCs in other taxa, such as *Polistes* paper wasps. In paper wasps, there has been debate about whether CHCs convey information about their bearer's fertility or dominance (Sledge et al. 2001, 2004; Dapporto et al. 2007). In theory, either is plausible, as both dominance rank and fertility are important parameters within social insect colonies. The indirect fitness of nestmates depends on the fecundity of the reproductive queen because colony reproduction is typically monopolized by one individual. As a result, workers are expected to cooperate with more fecund queens, so queens may be favored to signal their fertility to nestmates (Keller and Nonacs 1993). Signals of dominance may also be important. Rival assessment via signals can lower the costs associated with fighting (Maynard Smith 1982), so honest signals of agonistic ability are common in organisms with aggressive competition. In social insects, aggressive competition over rank is important, as rank determines reproductive success (Reeve 1991). In many species, potential queens fight to obtain the dominant, reproductive position (Roseler 1991); signals of agonistic ability may minimize the costs of colony conflict over rank. Dominance signals may be particularly important in taxa with multiple potentially reproductive individuals, such as primitively eusocial taxa with totipotent workers or colonies with multiple nest-founding queens.

An important step toward understanding how wasps use CHCs is to understand the precise information conveyed by CHCs. Questions about the information content of CHCs persist in paper wasps, in part, because much research has examined CHCs at times of the colony cycle when dominance and fecundity are inseparable (i.e., during the worker phase). For example, dominant and subordinate queens have different CHC profiles (Lorenzi et al. 1997; Dapporto et al. 2004), but the subordinate queen's ovaries quickly regress after the dominance hierarchy is established. Therefore, the CHC differences could be due to differences in either dominance or fecundity. Similarly, workers have different CHC profiles than queens (Sledge et al. 2001). Workers are subordinate to queens and also have reduced ovarian development. When a worker takes over the dominant, reproductive position on an orphaned nest, the worker's ovaries develop and her CHC profile becomes queen-like (Dapporto et al. 2005a). The change in worker CHC profile may reflect the worker's change in dominance status and/or ovarian development. However, dominance and fertility are not always correlated in paper wasps. High fertility is not required for a wasp to maintain her dominance status (Roseler and Roseler 1989). Further,

there is no relationship between dominance and fertility among queens prior to nest foundation (Reeve 1991, this study; but see Dapporto et al. 2006). Although the correlation between dominance and fertility during much of the colony cycle has produced confusion about the information conveyed by paper wasp CHCs, identifying the precise information conveyed by CHCs is an important step toward understanding how CHCs are used.

Here, we disentangle the effect of dominance and fertility on CHCs by examining CHC profiles among nest-founding queens of the primitively eusocial paper wasp, *Polistes dominulus* at the moment of nest foundation. *P. dominulus* have a flexible founding strategy, in which foundresses may either nest alone or in multi-foundress groups (Reeve 1991). When multiple foundresses cooperate, they have intense battles over dominance rank. After the battles, the loser becomes behaviorally subordinate and her ovaries regress (Roseler 1991). However, during the initial founding stage, there is variation in both agonistic ability and fertility (Roseler et al. 1984, 1985; Dapporto et al. 2005b), but there are no secondary effects of dominance on subordinate ovarian development at this early stage (Reeve 1991). Here, we confirm that ovarian development and dominance rank, as defined as a wasp's agonistic ability, are not correlated among early spring foundresses. Then, we test the relationship between CHCs, dominance, and fertility. We examine CHC profiles at nest initiation, during the time when foundresses engage in frequent, aggressive dominance, but there are no secondary, physiological effects of rank. If CHCs convey information about dominance, the ability of one individual to behaviorally dominate a rival, we predict that CHC profiles will be correlated with foundresses' agonistic abilities. If CHCs convey information about fecundity, we predict that CHC profiles will be correlated with ovarian development. In addition, we test how endogenous levels of juvenile hormone (JH) relate to CHCs and ovarian development. Because JH acts as a gonadotropin in *Polistes*, it may play an important role in mediating CHC profiles.

Methods

Wasp collection and dominance bouts

Over-wintered foundresses were collected during early May of 2006. Collections were made within days of nest initiation from Washtenaw County, Michigan. After collection, wasps were brought to the laboratory and paired by weight for dominance bouts. Pairs consisted of wasps collected from sites at least 1 mile apart to ensure they had no previous interactions.

Pairs were placed in an 8×8×2 cm clear plastic box (the fight arena), and allowed to battle for dominance in a 2-h videotaped bout. Each wasp in a pair was marked with Testors model paint on one wing for identification during a dominance bout. Aggressive behaviors, including biting, stinging, darting, and grappling were scored during the bouts. Dominance was established by mounting behavior. During a mount, the dominant wasp climbs on top of the subordinate while the subordinate crouches and lowers her antennae (West-Eberhard 1969). Mounts are commonly used to establish relative dominance (West-Eberhard 1969; Reeve and Nonacs 1997; Tannure-Nascimento et al. 2008) and mounting was always unidirectional. After dominance bouts, wasps were chilled, and then preserved for analysis of endogenous JH titers, CHC profiles, and ovarian development.

Cuticular hydrocarbon analysis

Wasp thoraces were kept frozen until used in CHC analysis. Thoraces were submerged in 500 µL of pentane for 10 min. After 10 min, the thorax was removed, and the pentane was dried under a nitrogen stream. The sample was re-eluted with 75 µL of heptane for analysis. One microliter of the sample was injected into a Thermo Finnigan Trace GC 2000, with a 25-m long DB-1 capillary column installed in a split injector (ratio is 1:100), with a 0.2 mm I.D. and a 0.33 micron film thickness. Each sample was run on the column for 40 min in a splitless mode. The temperature of the column was programmed from 80°C to 300°C, at 10°C min⁻¹. The column was held at 80°C for 3 min after injection, and the final temperature of 300°C was held for 30 min. Integration of peak areas was performed with Xcalibur. The GC was coupled to a Trace mass spectrometer, operated at 70 eV. Components were characterized by analysis of their mass spectra.

Juvenile hormone titer analysis

Endogenous JH-titers of 34 wasp pairs were measured. Immediately after each dominance bout, wasps were cooled, decapitated, and hemolymph was collected with baked microcapillary tubes. The amount of hemolymph collected from each wasp was measured (range=1.9–11.9 µl), then the hemolymph was stored in 500-µl chilled acetonitrile. JH was determined according to well established procedures used in honey bees (Huang et al. 1994; Jassim et al. 2000). Briefly, JH III in the hemolymph sample was extracted with 2×1 ml of hexane, then the pooled hexane extracts were evaporated using a vacuum centrifuge (Speedvac) linked to a condenser (Savant SS21), which trapped the solvent at -98°C. The dried JH in the sample tube was dissolved in 100 µl of methanol and a

20 µl aliquot (in duplicate) was taken out dried and mixed with 200 µl of buffer containing anti-JH antiserum (1:14,000 dilution, generous gift from David Borst) and 10,000 DPM of [³H(N)]-JH (Perkin Elmer, 647.5 Gbq/mmol). The mixture was incubated at room temperature for 2 h, then 0.5 ml of dextran-coated charcoal suspension (Sigma) was added to each sample tube to absorb the unbound JH. This mixture was incubated in ice-water mixture for 2.5 min, then centrifuged (2000×g for 3 min), and the supernatant, which contained bound JH, was decanted into scintillation vials. Liquid scintillation counting was performed using a Beckman LS 6500. A standard curve with various amounts (0, 3, 10, 30, 100, 300, 1,000, 3,000, and 10,000 pg) of standard JH (Sigma) was run each day. KaleidaGraph (Synergy Software, PA, USA) was used to generate a standard curve. The standard curve was obtained by using DPM bound as the dependent variable, JH amount (after log transformation) as the independent variable, and five unknown parameters, using nonlinear regression. The five-parameter formula was described in Huang and Robinson (1996). Excel (Microsoft, USA) was used to calculate the amount of JH in each sample, by reversing the five-parameter formula (solving for JH with known DPM and the five fitted parameters). TLC was used to verify that this method accurately assesses hemolymph JH-titer (Tibbetts et al., unpublished data).

Ovarian development

Wasp abdomens were stored in ethanol before dissection. The number and size of all eggs ≥1 mm in length were measured for each wasp.

Statistical analysis

Behavioral, ovarian, and cuticular hydrocarbon data were collected for 48 pairs of wasps (96 individuals). A few ovaries were not preserved for dissection, so the sample size for the ovarian development analyses was reduced to 45 pairs. Seven of the 48 pairs did not establish dominance during the bout, so the sample size for dominance analysis was reduced to 41. Juvenile hormone titers were obtained for 34 pairs of wasps, or 68 individuals. Sample sizes smaller than these represent comparisons in which two or more types of data were not obtained for a particular pair in the analysis. All analyses were run twice: once with the maximum sample size possible, and once including only those samples for which we had all four pieces of data (dominance behavior, CHCs, ovaries, and JH-titer). For all comparisons, results were similar across both analyses.

Cuticular hydrocarbon profiles were obtained for 48 pairs of wasps (96 individuals). 60 long-chain branched and

unbranched alkanes and alkenes were found in the wasps analyzed; compounds that appeared in less than 10% of the wasps were thrown out, and the remaining 35 compounds were used in the analysis. Peak areas for each compound were obtained using Xcalibur, and the resulting areas were standardized into percentages of the total peak area for a given profile. The 35 standardized compounds were reduced by principal components analysis. For win/loss analysis, the difference between peak areas within a pair was obtained before principal components analysis using Systat 9. For this paired (i.e., difference) data, the first 12 components explained 79.38% of the variance ($n=48$ profile pairs), and for the non-paired CHC data, the first ten components explained 76.86% of the variance ($n=96$ profiles). The resulting component scores were analyzed using multivariate statistics and backward stepwise regression in JMP In 5.1 and Systat 9, and all components used in the analyses had eigenvalues >1 . Paired data that was analyzed for dominance (i.e., win vs loss) was analyzed using paired logistic regression in JMP In 5.1. Differences in the data were calculated by subtracting the values of wasp 1 from the values of wasp 2; when wasp 1 was behaviorally dominant (i.e., the winner of the fighting bout), the pair was assigned a 1; if wasp 1 was behaviorally subordinate (i.e., the loser of the bout), the pair was assigned a 0. The resulting 1s and 0s were used as the response in the logistic regression model. Discriminant analysis on the non-paired dominance data was performed using JMP in 5.1.

Results

Cuticular compounds and dominance

There is no association between dominance rank and the cuticular hydrocarbon profile of emerged over-wintered foundresses. No significant differences in compound abundance were found between the cuticular hydrocarbon profiles of dominant and subordinate foundresses (logistic regression run on PCA component scores for components 1–12 for the paired data, $p=0.77$ for the model; no components were significant, $n=41$ pairs), and stepwise backward regression was not able to distinguish the two categories of foundress based on their hydrocarbon profiles (as no components were significant, this method failed to produce a model). Additionally, discriminant analysis (DA) performed on cuticular hydrocarbons failed to distinguish between dominant and subordinate foundresses, classifying only 81.71% correctly (100% of variation explained by Function 1, Wilks' lambda=0.53, $P=0.33$). Therefore, variation in the cuticular hydrocarbon profile cannot be attributed to dominance.

Cuticular compounds and ovarian development

There was a significant relationship between the cuticular hydrocarbon profile and the number of eggs in newly emerged foundresses. A general linear model using the non-paired PCA components one to ten revealed a significant relationship between cuticular hydrocarbon profiles and the number of eggs: $P=0.0099$, $n=90$, adj. $R^2=0.15$. To create a more parsimonious model, stepwise backward regression was used and revealed a significant correlation with components 1, 2, 4, 5, 6, 8, and 10, $P=0.0042$, adj. $R^2=0.15$, $n=90$. There was also a significant relationship between the CHC profile and the average egg area. Stepwise backward regression run on the non-paired PCA components one to ten revealed a significant model using components one and five: $P=0.0196$, adj. $R^2=0.065$, $n=90$. Therefore, CHC profiles are correlated with fertility. Wasps differing in their fecundity, measured as the number and average size of eggs, have very different CHC profiles.

Dominance and ovarian development

There was no relationship between fecundity and dominance. Foundresses with larger or more numerous eggs were not more likely to become dominant than those with smaller, or fewer numbers of eggs. Dominance vs. difference in number of eggs: $P=0.968$, $n=39$; paired logistic regression (Fig. 1a); dominance vs. difference in average egg area: $P=0.715$, $n=39$; paired logistic regression (Fig. 1b).

Relationships with juvenile hormone

There is a significant relationship between juvenile hormone titers and the CHC profile. A general linear model using the non-paired PCA component scores for components one to ten revealed a significant relationship between CHCs and JH-titer: $P=0.002$, adj. $R^2=0.35$, $n=52$ (of the 34 pairs of wasps with JH titers measured, 26 pairs also had their CHC profiles analyzed). Stepwise backward regression was performed to create a more parsimonious model. The results revealed a significant relationship between JH-titer and PCA component scores for components 3, 4, 5, 6, 8; $P<0.0001$, adj. $R^2=0.4$, $n=52$ (JH was log-transformed to obtain a normal distribution). Therefore, wasps with varying levels of endogenous juvenile hormone are chemically distinguishable.

Endogenous levels of juvenile hormone correlated significantly with the number of eggs, such that higher levels of JH corresponded to a larger number of eggs: $P<0.0001$, $R^2=0.24$, $n=64$ (standard least squares analysis; JH was log-transformed to obtain a normal distribution), see

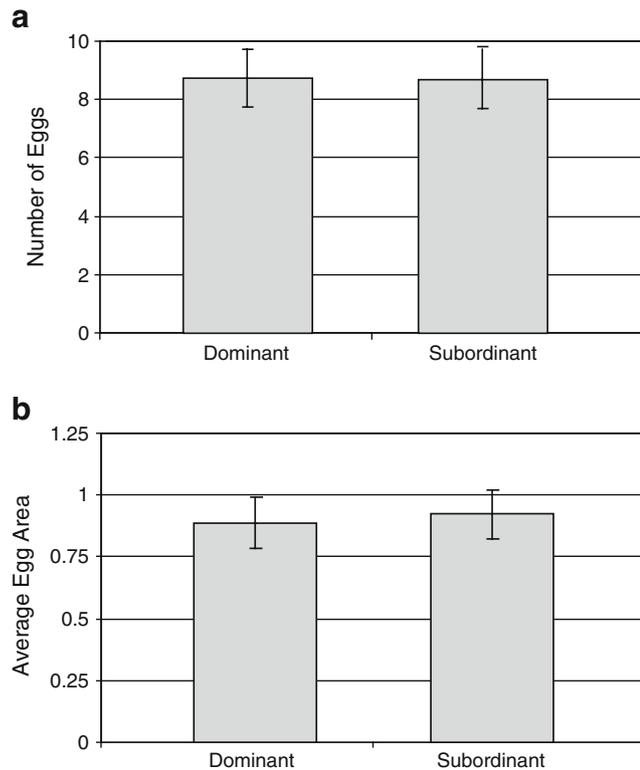


Fig. 1 **a** Bar graph representing the means and standard errors of the number of eggs present in dominant and subordinate foundresses. Dominant foundresses do not possess more eggs than subordinate foundresses. **b** Bar graph representing the means and standard errors for the average egg area of dominant and subordinate foundresses. Dominant foundresses do not possess larger eggs than subordinate foundresses

Fig. 2a. Additionally, there was a significant relationship between the average area of the eggs and levels of JH: $P=0.015$, $R^2=0.077$, $n=64$ (general linear model; JH was log-transformed to fit the assumptions of normality), see Fig. 2b. Juvenile hormone may be the mechanism which links cuticular compounds to fertility in emerging overwintered foundresses.

Because relationships were found between both JH and CHC profiles, and ovarian development and CHC profiles, a MANOVA was run to determine the relative contributions of each to chemical profile expression. The whole model, including both JH and ovarian development as dependent variables was significant (whole model: Wilk's Lambda=0.3416, $F(10,36)=2.559$, $P=0.0019$). However, this is mainly attributed to the relationship between CHCs and JH (JH term: $P=0.0006$, $F=4.303$) rather than the relationship between CHCs and ovarian development ($P=0.3238$, $F=1.1995$). Therefore, the relationship between CHCs and ovarian development may be a secondary effect of the relationship between CHCs and JH.

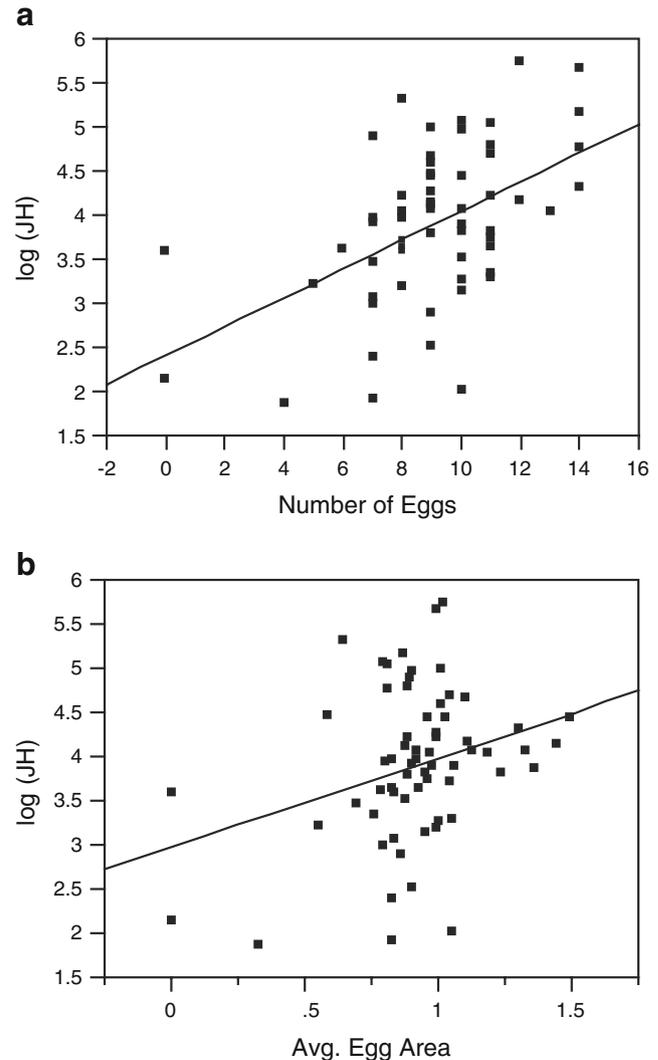


Fig. 2 **a** Relationship between juvenile hormone titer and number of eggs. Foundresses with more eggs possessed higher levels of endogenous JH. **b** Relationship between juvenile hormone titer and average egg area. Foundresses with larger eggs have higher levels of endogenous JH than foundresses with smaller eggs

Discussion

There has been long-standing interest in the information conveyed by cuticular hydrocarbons, with particular debate over whether CHCs are signals of dominance or fertility. Controversy has persisted in paper wasps, in part, because most research has been performed during times of the colony cycle when fertility and dominance rank are coupled. Here, we examine CHC profiles at the time of nest foundation, when differences in ovarian development and agonistic ability are due to individual characteristics rather than rank within a nest. We found a significant relationship between CHC profiles and fertility. Foundresses with more eggs have different CHC profiles than those with fewer eggs. However, there was no relationship

between CHC profiles and dominance rank. As a result, CHCs could convey information about their bearer's fertility, but are unlikely to be signals of dominance.

CHCs have long been hypothesized to convey information about their bearer's fertility. Support for the relationship between CHCs and fertility largely comes from studies showing that queens and workers (i.e., reproductive and non-reproductive individuals) have distinct CHC profiles, as do queens of differing reproductive ranks (in ants: Monnin et al. 1998; Peeters et al. 1999; Cuvillier-Hot et al. 2001; Heinze et al. 2002; Tentschert et al. 2002; Dietemann et al. 2003; de Biseau et al. 2004; Endler et al. 2004; in bees: Ayasse et al. 1995; Bloch and Hefetz 1999; in wasps: Bonavita-Cougourdan et al. 1991; Butts et al. 1995; Sledge et al. 2001). The strength of this relationship across taxa is striking. However, queens and workers differ in more than just their fertility, so these broad correlations do not provide information about the specific information conveyed by CHCs. In ants, there is solid evidence that CHCs function as fertility signals: particular chemicals have been identified that are consistently correlated with reproductive status (D'Ettore et al. 2004), manipulation of fertility levels reliably changes the CHC profile of manipulated ants (Cuvillier-Hot et al. 2004b), and application of these compounds on non-reproductive workers initiates worker policing (Smith et al. 2009). Here, we provide compelling evidence that CHC profiles also provide information about fertility in wasps, as has been proposed by previous authors (Bonavita-Cougourdan et al. 1991; Sledge et al. 2001, 2004).

Although there is a general relationship between CHCs and fertility, less is known about the extent of fertility information that CHCs convey. In particular, are CHCs a graded signal of fertility or do they merely convey information about whether or not a female is capable of laying eggs? A queen's fertility is an important parameter within social insect colonies, as a queen with low fecundity could be disastrous for colony success. Consequently, many large colony social insects use chemical information to establish that the queen is present and laying eggs (Beekman 2004; Endler et al. 2004). Although small colony social insects are thought to lack the specialized signals of fertility found in more advanced eusocial taxa, CHCs could potentially fulfill this role.

Our data indicate that CHCs may function as graded fecundity signals. Among fertile females, individuals with more eggs have different CHC profiles than those with fewer eggs. A similar pattern has been found in both monogynous queenless ants and ponerine ants, in which CHC profiles reflect the extent of gamergate and queen ovarian development (Liebig et al. 2000; Cuvillier-Hot et al. 2004a). Therefore, CHC profiles do not operate in an "all or nothing" manner; rather CHC profiles may convey

information about a gradient of fecundity levels. Future work examining the relationship between fecundity and CHCs is important to establish whether CHCs are graded signals of fertility across taxa. Given the importance of fertility in the behavioral interactions of social insects, such a ubiquitous fertility signal could be a key parameter in colony dynamics from queen assessment to worker policing.

Numerous studies have suggested that there is a link between CHCs and dominance, but these studies largely rely on the same correlations used to support the relationship between CHCs and fertility (Monnin et al. 1998; Monnin and Peeters 1999; Dapporto et al. 2007; Jackson 2007). Sledge et al. (2001) used the correlation between CHCs and dominance in the early versus late founding stage to conclude that CHCs are more likely to signal fertility than dominance in *P. dominulus* wasps. However, Dapporto et al. (2007) came to a different conclusion. They tried to disentangle dominance and fertility by experimentally increasing foundress egg-laying rate by emptying nest cells. Both alpha and beta queens laid replacement eggs, but the alpha and beta still had different CHC profiles. The authors concluded that the persistent difference in CHC profiles should be attributed to differences in foundress dominance rank. However, the alpha still had a higher egg-laying rate than the beta, so the difference in CHCs could also be caused by fertility differences. Therefore, most evidence suggests that CHCs in paper wasps convey information about their bearer's fertility, although social circumstances may have an impact on CHC profile expression.

Given the correlations between CHCs and fertility, but not CHCs and dominance, how are CHCs likely to be used within paper wasp colonies? Over its life, a paper wasp interacts with individuals that have many different interests. During the early spring, foundresses battle with many rivals before they found a nest, so information about agonistic ability is likely to be particularly important (Roseler 1991). Nevertheless, we found no relationship between CHC profiles and wasp's ability to dominate rivals during this period, suggesting that CHCs cannot convey useful information about their bearer's dominance potential. Instead, *P. dominulus* foundresses may rely on visual signals of agonistic ability to assess the abilities of unfamiliar rivals (Tibbetts and Dale 2004; Tibbetts and Lindsay 2008). Before nest founding, fertility information may not be particularly important, so wasps may not pay close attention to the fertility information that CHCs convey. However, CHCs are likely to be essential later in the colony cycle, when fertility is a key parameter within colonies. The information CHCs convey about individual fertility may be used to identify the queen, assess her fertility, and identify workers with developed ovaries (Bloch et al. 2000; Liebig et al. 2000; Heinze et al. 2002;

Cuvillier-Hot et al. 2004a). Therefore, visual and chemical signals convey different information and are likely to be important in different contexts within wasp nests.

In addition to providing compelling support for the fertility-signaling hypothesis, our results also suggest that JH may be the mechanism underlying chemical fecundity signaling. We found a significant relationship between the level of circulating JH and degree of ovarian development, such that more fecund wasps had higher endogenous JH titers than less fecund wasps. Additionally, JH titers were correlated with CHC profiles. Therefore, JH could mediate the relationship between CHCs and fertility. Juvenile hormone is a gonadotropin and has been termed the “pleiotropic master hormone” for its many important functions in insects (Hartfelder 2000). It integrates physiology, behavior, and endocrinology, and has many functions, including orchestrating metamorphosis, regulating female fertility, generating polymorphisms or polyethisms, controlling pheromone production, and influencing aggressive behavior (Robinson and Vargo 1997; Hartfelder 2000). JH also contributes to dominance in *Polistes* (Roseler 1991; Tibbetts and Izzo 2009; Tibbetts et al., unpublished data). However, the relationship between JH and dominance is complicated, as hormonal effects are both condition- and context- dependent (Tibbetts and Izzo 2009, Tibbetts et al., unpublished data). Further manipulative studies are necessary to clarify the relationships between JH, dominance, fertility, and chemical profile expression, and to determine if JH directly influences CHC production.

Previous work on JH in wasps has largely relied on correlations between the size of the corpora allata (CA; the organ that synthesizes JH), the JH biosynthesis rate, dominance, and level of ovarian development (Bohm 1972; Roseler et al. 1984; Roseler et al. 1985; Larrere and Couillaud 1993; Bloch et al. 2000; Agrahari and Gadagkar 2003; Sledge et al. 2004; Brent et al. 2005; Giray et al. 2005; Lengyel et al. 2007). These data have produced some interesting relationships. For example, Sledge et al. (2004) found a relationship between CHCs and CA size. However, JH biosynthesis rate and CA size do not always correlate with circulating JH titer (Lanzrein et al. 1978; Bloch et al. 2000). Our data is among the first to show direct relationships among endogenous (circulating) measures of JH, fecundity, and cuticular hydrocarbon profiles. We found a significant link between fecundity, hormone levels, and CHC profile.

CHCs are ubiquitous among social insects and provide an important behavioral mechanism for mediating colony dynamics. Our results indicate that CHCs may function as graded signals of their bearer's fertility, but do not convey useful information about their bearer's dominance. JH titers are strongly associated both with fertility and CHC profiles, suggesting that JH may be the hormonal mechanism underlying variation in CHC profiles. CHCs are complex,

multifaceted signals that are difficult to experimentally manipulate, so many questions remain. Nevertheless, fertility signaling offers an evolutionarily stable strategy for maintaining cooperation within a nest after worker emergence. Our data both provide support for this hypothesis as well as a potential mechanism of chemical fertility signaling.

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