Differences in Long-Chained Cuticular Hydrocarbons between Males and Gynes in *Cataglyphis* Desert Ants

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Abstract

Cuticular hydrocarbons play an important role in chemical communication in social insects, serving, among other things, as nestmate, gender, dominance and fertility recognition cues. In ants, however, very little is known about the precopulatory signals cuticular hydrocarbons carry. These signals may serve as affecting sex pheromones and aphrodisiacs or as reliable signals for idiosyncratic traits, which indirectly affect sexual selection. In this study, we examined, for the first time in the *Cataglyphis* genus, sex-specific variability in cuticular hydrocarbons. We focused on a population in which we observed either unmated queens or males, but never both, present in the same colony before mating season. We found significant quantitative differences, but no qualitative differences, between virgin queens and their potential mates. In analyses of both absolute amounts and relative amounts of cuticular hydrocarbons, we found different compounds to be significantly displayed on gynes and drones, suggesting absolute and relative amounts may carry different signals influencing mating behavior and mate choice. We discuss the possible signals advertised by the non-polar fraction of these hydrocarbon profiles.

Key Words- Cataglyphis niger, Sexual selection, Sex-specific cuticular hydrocarbons.

INTRODUCTION

While in most animals sexual selection presents itself in many elaborate and colorful ways, in social insects, pre-mating sexual selection is considered to be limited (Boomsma *et al.* 2005). And still, there are various mechanisms through which sexual selection acts that have been demonstrated in social insects, and in ants specifically, most of

which are post-mating, such as sperm selection, use of mating plugs, and increased mating frequencies by both males and females (see overview in Boomsma *et al.* (2005)). Precopulatory selection in ants, however, is limited to few cases of male territoriality (Abell *et al.* 1999; Davidson 1982; Heinze *et al.* 1998; Kinomura and Yamauchi 1987; Stuart *et al.* 1987; Wiernasz *et al.* 2001) and semiochemical communication, which has been surprisingly understudied (Ayasse *et al.* 2001).

Some studies have shown virgin queens to be advertising sexual receptivity (Reviewed in Ayasse *et al.* (2001) and Hölldobler and Wilson (1990)) and others have identified active female sex pheromones in several species (*Formica lugubris* in Walter *et al.* (1993), *Polyergus breviceps* in Greenberg *et al.* (2007) and in Greenberg *et al.* (2018), *Polyergus rufescens* in Castracani *et al.* (2005) and in Castracani *et al.* (2008)). Mandibular, Dufour, poison, and pygidial glands have also been shown to contain attractants to drones (Grasso *et al.* 2003). In several species, drones have been shown to discharge their mandibular glands during nuptial flights or when leaving the nest (BENTO *et al.* 2007; Brand *et al.* 1973b; Hölldobler 1976; Law *et al.* 1965) and male metapleural glands were also suggested to have an active role in sexual selection (Hölldobler and Engel-Siegel 1984).

Cuticular hydrocarbons (CHCs) are likely to play an important role in mating behavior and mate choice in ants because many other individual and colonial difference such as task (see for example Greene and Gordon (2003)), caste and sex (see for example Campos *et al.* (2012)), nestmate recognition (See for example Lahav *et al.* (1999)) and fertility (See for example Dietemann *et al.* (2003)) are advertised by the cuticular chemical coating. Part of the chemical bouquet displayed on the cuticle may be active sex pheromones, but it can also contain signals indicative of other traits which influence mating behavior. In ants, few studies demonstrated unique sex-specific differences in CHCs between virgin queens (hereafter gynes) and males (drones). Those studies showed these differences to be either qualitative or quantitative (Antonialli Junior *et al.* 2007; Brand *et al.* 1973a; Chernenko *et al.* 2012; Cremer *et al.* 2002; Cuvillier-Hot *et al.* 2001; Hojo *et al.* 2008; Johnson and Sundström 2012; Kureck *et al.* 2011; Oppelt *et al.* 2008).

To the best of our knowledge, no study examined sexual dimorphism in CHCs in *Cataglyphis* ants and the mechanisms underlying sexual selection are still unknown in this large genus. In *C. iberica* a mixture of three linear alkanes and methylalkanes (n-C27, nC29 and 3-meC29) has been suggested to function as queen pheromones but their function was restricted to sterility inducement rather than male attraction (Van Oystaeyen *et al.* 2014). Another

study found that gynes produce more undecane in the Dufour's gland than drones (Monnin *et al.* 2018) but also suggested it may have functions unrelated to male attraction.

Another possible mechanism related to precopulatory sexual selection may involve altering acceptance threshold making drones able to get closer to gynes without being harassed by workers (Helft *et al.* 2016; Helft *et al.* 2015). There is, indeed, evidence of drones mimicking the queen's chemical bouquet in ants, thus allowing them to escape aggression from other drones (Cremer *et al.* 2002) or from workers (Franks and Hölldobler 1987). Other such acceptance-altering effects have been demonstrated in honeybees where different nestmate recognition mechanisms are used to identify drones and workers (Moritz and Neumann 2004), enabling drones to immigrate to foreign colonies.

Kinship between gynes and drones may also influence mate choice. Although it has been argued that discriminatory abilities are usually limited to nestmate recognition, regardless of kinship degree (Carlin 1988; Grafen 1990), there is evidence of the influence of kinship on mating preferences in ants (Keller and Passera 1993). Keller and Passera (1993), in this study, suggested that colony-derived cues may be of less importance in mating preferences than kinship cues. In bees, male have also been shown to discriminate between female kin through olfactory signals, such as CHCs (Smith 1983) suggesting that kinship recognition is, indeed, of importance in mate choice.

CHC may carry other signals which affect pre-mating sexual selection in ants such as overall fitness of both gynes and drones. Body size, in both sexes, is a reliable indicator of fitness and, in *Pogonomyrmex* harvester ants, for example, larger drones have been documented to be more successful in mating attempts (Abell *et al.* 1999; Wiernasz *et al.* 2001; Wiernasz *et al.* 1995). In ants, gynes and drones vary significantly in body size, ranging from both sexes being about equal in size to queens having ∼3 times the body length and ∼25 times the body mass of males (Boomsma *et al.* 2005). Cataglyphis ants show significant variability in body size between gynes and drones.

Body size and surface area effect the absolute amounts of CHCs coating an individual ant and the total amount of a component is proportional to it. It is hard to determine whether a factor in the pheromonal blend is informative because of its absolute amount, its ratio with other chemicals, or both factors combined. Ratios between CHCs are usually less variable than absolute quantities of each compound (See for example in Drosophila Coyne (1996)) because absolute amounts are affected by body size. The opposite case was also reported, with higher variability in

relative amounts than in total amounts (See for example, also in Drosophila, Grillet *et al.* (2005)). Thus, body size variation of both gynes and drones may play a role in mating. We thus chose a twofold analysis of the CHCs to account for the possibility of absolute amounts enhancing differences between gynes and drones in ants of *C. niger* and also the possibility of them masking differences. To our knowledge, this is the first study to demonstrate sexual dimorphism in CHCs in *Cataglyphis* and it suggests that ratios between compounds may carry different signals than absolute amounts.

METHODS AND MATERIALS

Ants

Ants were collected between April 24 and May 10, 2016 from colonies dug along a 4 km transect in Betzet beach on the northern Israeli coastline (from N33.05162, E35.10245 to N33.07868, E35.10705). This population was previously referred to as *Cataglyphis drusus* (Eyer *et al.* 2017) but our recent species delimitation study suggested that this is the same species as *C. niger*, because these populations are not differentiated by their nuclear genomic DNA (Brodetzki *et al.* 2019). Colonies of this population are monogyne (headed by a single queen) and polyandrous (queens are multiply mated) (Brodetzki *et al.* 2019; Eyer *et al.* 2017). Queens usually mate during the spring and sexuals (gynes and drones) can usually be found in nests in April-May. In this study, on the days when ants were collected, we observed colonies with either gynes or drones (or neither of the two) present in the colony before mating season. We used samples from twelve nests, 6 female-producing colonies and 6 male-producing colonies, with 1-3 gynes/drones collected from each colony. A total of 13 gynes and 10 drones were analyzed. All sexuals were frozen on the same evening of collection.

Cuticular hydrocarbon analysis:

Whole bodies were individually immersed in hexane, containing 400 ng/µl of tetracosane (C24) as internal standard for quantitative analysis. Initial analysis was conducted by gas chromatography/mass spectrometry (GC/MS), using a VF-5ms capillary column, temperature-programmed from 60◦C to 300◦C (with 1 min initial hold) at a rate of 10◦C

per min, with a final hold of 15 min. Compound were identified according to their fragmentation pattern and respective retention time compared to authentic standards. We identified 34 compounds in gynes and the same 34 compounds in drones. Quantitative analyses for each individual were performed by flame ionization gas chromatography (GC/FID), using the above running conditions. Peak integration was performed using the program Galaxie Varian 1.9.

Data and Statistical analysis

Statistical analysis was performed using XLSTAT (https://www.xlstat.com; Addinsoft 2019;Boston, USA).

RESULTS

Chemical analysis of the non-polar fraction of the cuticular extracts of gynes and drones identified 34 long-chained CHCs, ranging from pentacosane (c25) to tritriacontane (c33). All 34 compounds were identified in both gynes and drones with no qualitative differences between them (Fig. 1). Quantitative differences between the two groups were examined in two ways: first, an analysis of absolute quantities calculated relative to an internal standard; second, an analysis of the percentage of every compound in the total extract, calculated according to percent area in peak integration (Table 1). Principal component analyses (PCA) of both measures are shown in Figure 2. Colony level variation did not seem to mask the differences between gynes and drones' cuticular profiles as shown in Figure 2.

In a linear discriminant analysis (LDA), both relative amounts and absolute quantities showed significant differences between CHC profiles of gynes and drones (In Wilks' Lambda test (Rao's approximation) *p*-values were 0.044 and 0.002, for absolute amounts and relative amounts respectively). Relative amounts were less variable than absolute amounts. Following an LDA analysis, a unidimensional test of equality of the means of the groups showed different compounds to be significantly discriminating between gynes and drones in the two analyses; four compounds in the relative amount analysis: C25; 11,15-dime C29; 2-me C30; C31; and 13 compounds in the absolute amounts analysis: 5-me C25; 3-me C25; 10-me C26; 11+13-me C27; 11,15-dime C27; 3-me C27; C28; 11+13-me C29; 3-

me C29; 7,11,15-trime C29; C30; 4-me C30; 13+15-me C31. Only one compound was identified in both analyses - 7,11,15-trime C27 (Table 1).

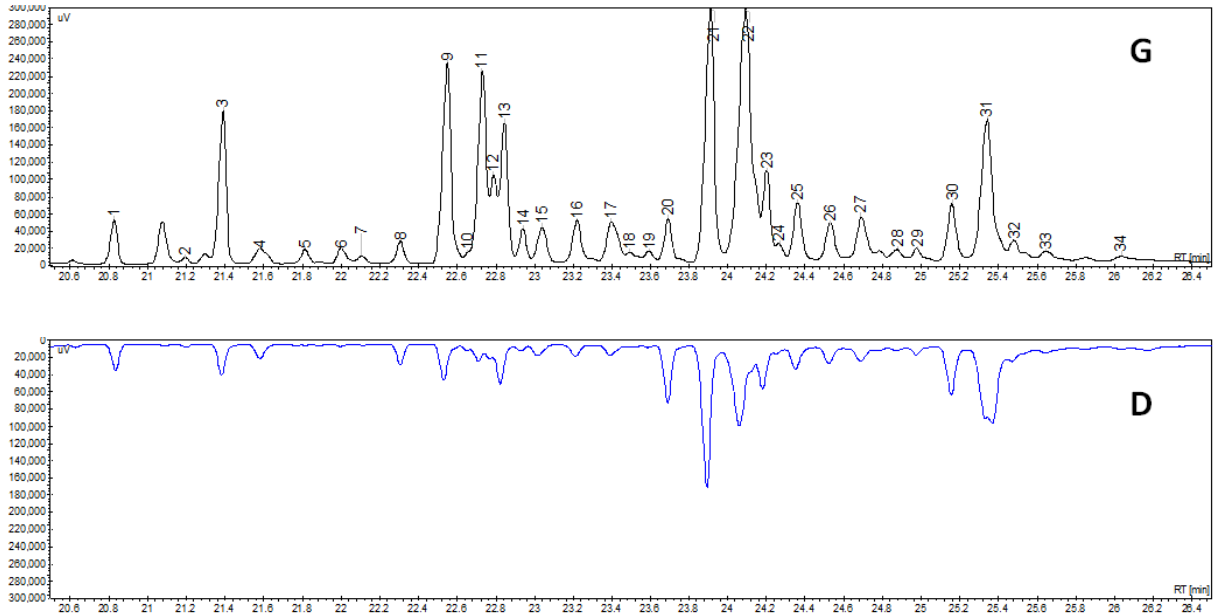


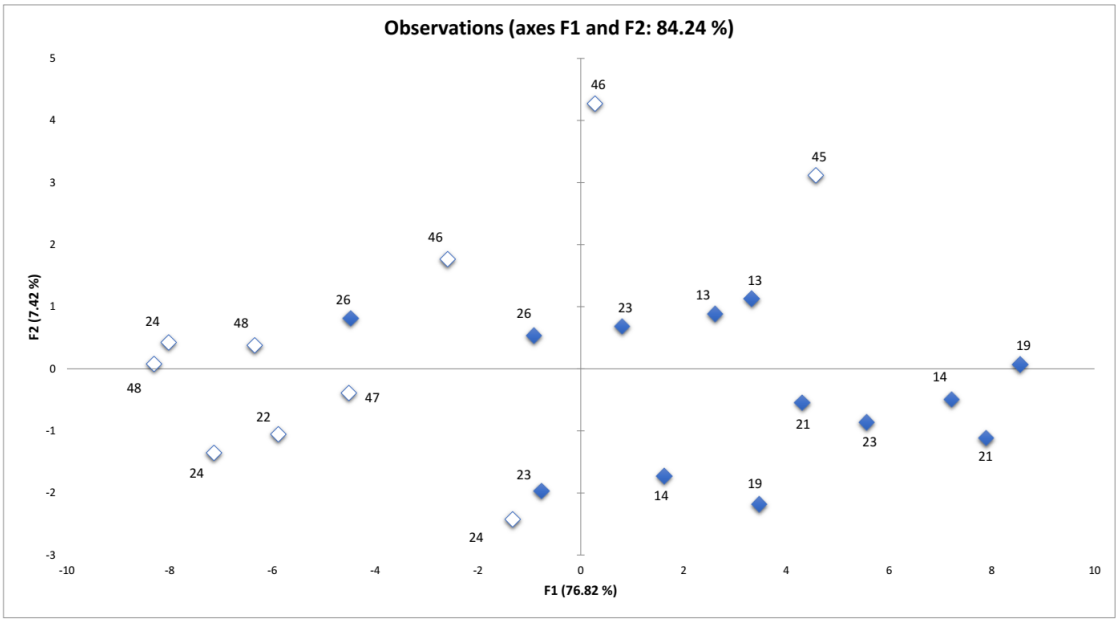
Figure 1: Chromatograms of CHCs from total body extracts. The upper chromatogram is of a gyne and the lower of a drone. Only long-chained CHC are shown.

Table 1: Quantification of CHCs of 13 gynes (from 6 colonies) and 10 drones (from 6 colonies)

		Gynes Absolute quantity (ng)	Drones Absolute quantity (ng)	P- Value	Gynes % of total secretion	Drones % of total secretion	P- Value
1	c25	1169.10±780.33 (239.73 - 3288.89)	865.49±1345.39 (78.36 - 4512.50)		1.80±0.66 (1.14 - 3.45)	2.74±0.93 (1.37 - 3.96)	0.010
2	5me c25	108.92±104.41 (24.66 - 400.00)	69.43±69.84 (1.51 - 237.50)	0.046	0.15±0.06 (0.07 - 0.27)	0.38±0.42 (0.01 - 1.40)	
3	3me c25	4040.73±4316.35 (528.77 - 17266.67)	1083.44±879.80 (181.29 - 3112.50)	0.006	5.27±1.65 (3.37 - 8.14)	5.17±2.73 (2.49 - 9.12)	
4	c26	768.13±363.13 (363.01 - 1577.78)	311.59±333.96 (31.83 - 912.94)		1.35±0.59 (0.71 - 2.67)	1.21±0.59 (0.49 - 2.23)	
5	10me c26	286.50±373.59 (17.81 - 1422.22)	35.19±33.87 (5.26 - 115.94)	0.024	0.33±0.18 (0.10 - 0.64)	0.22±0.18 (0.02 - 0.46)	
6	4me c26	401.26±408.87 (67.12 - 1622.22)	83.34±61.54 (9.00 - 187.50)		0.53±0.13 (0.35 - 0.73)	0.42±0.26 (0.14 - 0.86)	
7	4,12 dime c26	171.95±161.97 (20.55 - 622.22)	44.34±38.63 (7.61 - 137.50)		0.22±0.06 (0.15 - 0.30)	0.20±0.10 (0.11 - 0.43)	
8	c27	822.32±408.46 (298.63- 1844.44)	1170.72±2631.94 (84.43 - 8587.50)		1.38±0.43 (0.68 - 2.20)	2.33±1.70 (0.79 - 6.89)	
9	11+13 me c27	6150.91±6536.77 (702.74- 25200.00)	1425.94±1218.75 (147.40 - 4100.00)	0.038	7.72±2.67 (4.72 - 11.36)	6.49±3.42 (2.27 - 11.21)	
10	5me c27	70.74±56.68 (11.94- 222.22)	26.88±30.06 (0.92 - 100.00)		0.12±0.08 (0.02 - 0.28)	0.12±0.08 (0.01 - 0.26)	
11	11,15 dime c27	2254.88±3164.19 (95.89 - 11311.11)	373.00±415.91 (3.46 - 1405.80)	0.011	2.44±2.09 (0.69 - 6.54)	2.00±2.16 (0.06 - 5.32)	
12	7,11 dime c27	475.86±448.06 (60.27 - 1785.71)	73.76±65.60 (4.84 - 220.38)		0.72±0.41 (0.15 - 1.51)	0.44±0.42 (0.07 - 1.50)	
13	3me c27	2017.75±1746.58 (445.21 - 7133.33)	924.67±1204.11 (131.58 - 4137.50)	0.004	2.85±0.55 (1.85 - 3.39)	3.31±0.66 (2.42 - 4.43)	
14	7,11,15 trime c27	675.95±521.63 (83.56- 1888.89)	124.08±100.80 (6.23 - 312.50)	0.021	0.93±0.18 (0.62 - 1.32)	0.63±0.36 (0.10 - 1.02)	0.016
15	c28	1327.92±956.33 (301.37- 3666.67)	470.54±589.69 (71.97 - 2062.50)	0.011	1.93±0.21 (1.65 - 2.36)	1.76±0.39 (1.11 - 2.47)	
16	12me c28	1652.54±1337.81 (272.60- 5044.44)	421.62±395.33 (59.52 - 1387.50)		2.27±0.34 (1.81 - 3.14)	1.83±0.65 (0.92 - 2.94)	
17	4me+ 8,12 dime c28	1701.79±1448.09 (304.11- 5666.67)	393.80±344.16 (56.75 - 1187.50)		2.31±0.18 (2.01 - 2.65)	1.79±0.65 (0.87 - 2.58)	
18	2me c28	60.12±45.04 (14.93- 155.56)	25.03±33.88 (3.46 - 100.00)		0.09±0.04 (0.04 - 0.15)	0.08±0.03 (0.05 - 0.16)	
19	4,12 dime c28	200.57±162.33 (32.88- 577.78)	32.42±25.33 (9.69 - 98.55)		0.28±0.04 (0.22 - 0.35)	0.20±0.11 (0.02 - 0.34)	
20	c29	1350.87±663.95 (334.38- 2444.44)	3601.76±8320.86 (155.76 - 26875.00)		2.36±1.16 (0.90 - 4.50)	6.09±6.17 (1.21 - 21.57)	
21	11+13 me c29	12893.58±9321.57 (2461.64- 35444.44)	5024.77±6563.04 (803.51 - 22350.00)	0.014	18.38±1.78 (15.05 - 21.01)	17.31±1.98 (14.32 - 20.82)	
22	11,15 dime c29	8757.70±6757.88 (66.67- 26628.57)	2583.85±2871.68 (532.75 - 10000.00)		14.38±5.04 (0.03 - 19.07)	10.30±2.02 (7.21 - 14.51)	0.025
23	3me c29	4347.20±10298.52 (575.00- 38511.11)	864.98±981.86 (182.46 - 3000.00)	0.014	3.86±4.14 (1.57 - 17.36)	3.26±0.78 (2.30 - 4.96)	
24	7,11,15 trime c29	96.00±86.39 (6.25 - 314.29)	20.99±18.17 (4.61 - 66.67)	0.025	0.13±0.06 (0.02 - 0.22)	0.12±0.07 (0.02 - 0.23)	
25	c30	2255.41±1856.09 (494.52 - 6977.78)	712.55±873.58 (178.55 - 3050.00)	0.018	3.10±0.46 (2.04 - 3.78)	2.73±0.40 (2.15 - 3.38)	
26	14 me c30	1755.93±1438.75 (287.67 - 5355.56)	514.17±593.81 (141.94 - 2075.00)		2.37±0.25 (1.93 - 2.67)	2.06±0.48 (1.46 - 2.72)	
27	4 me c30	1773.93±1566.14 (353.42 - 5911.11)	432.60±407.25 (145.62 - 1437.50)	0.017	2.37±0.36 (1.62 - 2.79)	1.95±0.52 (1.15 - 2.70)	
28	2 me c30	311.34±321.04 (63.01 - 1177.78)	45.75±33.89 (18.43 - 113.04)		0.40±0.10 (0.21 - 0.56)	0.26±0.13 (0.07 - 0.45)	0.011
29	c31	276.17±165.32 (31.25- 566.15)	562.24±1278.31 (19.35 - 4150.00)		0.51±0.36 (0.10 - 1.17)	1.14±0.91 (0.16 - 3.33)	0.035
30	13+15 me c31	3413.97±2341.85 (709.59- 8400.00)	2003.26±3378.76 (251.61 - 11262.50)	0.019	4.96±1.15 (3.05 - 6.66)	6.12±2.88 (2.85 - 10.66)	
31	11,15 dime c31	9198.67±6728.53 (2210.96 - 24933.33)	3385.37±3031.83 (985.26 - 9477.65)		13.32±3.24 (7.79 - 18.57)	16.09±7.76 (6.15 - 30.50)	
32	7,11,15 trime c31	439.66±366.76 (65.63 - 1333.33)	95.61±106.64 (18.43 - 375.00)		0.62±0.20 (0.21 - 0.89)	0.50±0.33 (0.07 - 1.02)	
33	c32	392.66±421.94 (41.18 - 1400.00)	133.82±114.07 (23.96 - 375.00)		0.49±0.24 (0.07 - 0.86)	0.69±0.49 (0.28 - 1.75)	
34	c33	8.60±11.22 (0.00 - 42.86)	20.65±34.13 (0.00 - 112.50)		0.05±0.11 (0.00 - 0.41)	0.08±0.11 (0.00 - 0.37)	

Means ± Standard deviation are shown and ranges are given in parentheses.

(A)



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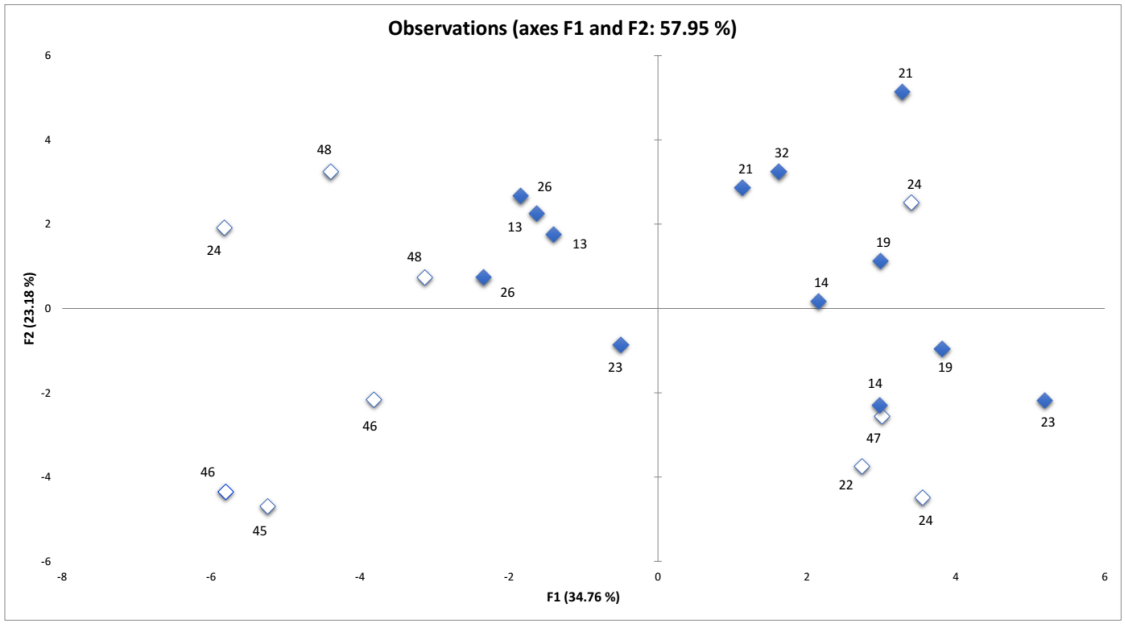

Figure 2: Principal component analysis (PCA). **(A)** Absolute quantities **(B)** Percent of total extract. Filled blue points represent individual observations of gynes and empty points of drones. Numbers indicate colony identity.

DISCUSSION

Our study reveals differences in long-chained CHCs between drones and gynes in *Cataglyphis niger* colonies. The differences were quantitative and not qualitative and different compounds stand out in a discriminant analysis when considering either relative amounts or absolute amounts. The two analyses revealed different compounds to be significantly discriminating virgin queens and their potential mates.

We reported that on the days when ants were collected, we observed colonies with either gynes or drones (or neither of the two) which may suggest that this species exhibit a split sex ratio (Boomsma 1991; Boomsma and Grafen 1990, 1991; Bourke 2005; Chan and Bourke 1994; Chapuisat and Keller 1999; Grafen 1986; Mehdiabadi *et al.* 2003; Pamilo and Seppä 1994; Queller and Strassmann 1998; Ratnieks and Boomsma 1997; Sundström 1994). As (Trivers and Hare 1976) suggested, split sex-ratio may be the result of a tug-of-war between queens and workers over reproductive control. There is reason to believe that this is the case in our population because previous studies suggest that in colonies with singly-mated queens, queens favor a 1:1 ratio of gynes to drones and workers a 3:1 ratio. Although studies from singly-mated species revealed frequent female-biased sex ratios indicating that workers often prevail (Herbers 1984; Nonacs 1986; Trivers and Hare 1976), the queen may thwart worker control by specializing in producing either gynes or drones. However, determining whether colonies exhibit split sex-ratios requires a long-term study. It is possible that colonies alternate between female and male production in different years or that the production is temporally offset, so that drones are produced slightly before gynes or vice versa (Keller *et al.* 1996). Colonies, therefore, should be thoroughly examined at different times in the reproductive season and in multiple years. We have not done so in the present study because our goal was different: to determine differences in circular hydrocarbons between gynes and their potential mates as a potential mechanism involved in sexual selection.

We reported using 1-3 gynes or drones from each of 6 female and 6 male producing colonies. This sample size may seem small in light of the number of variables (34 compounds) that were identifies, however because CHC's are produced in series (Martin and Drijfhout 2009), the number of variables is, in fact, smaller and a large sample size is not necessary in order to determine statistical significance.

Interestingly, when results were compared to previous studies in ants, similar discriminatory compounds could be found. The three linear alkanes and methylalkanes found in *C. iberica* (Van Oystaeyen *et al.* 2014) were also present in *C. niger* but only 3-meC29 was statistically different between gynes and drones in both species. This difference was only found in our absolute amounts analysis and not in the relative amounts analysis. Six CHC's which were identified in our analysis were also identified in *Formica fusca* (Chernenko *et al.* 2012) (3-MeC25, 4-MeC26, 11,15- diMeC27, 3-MeC27 12-MeC28, 8,12-diMeC28, C29) but only three of them were statistically different between gynes and drones in both species (3-MeC25, 11,15- diMeC27, 3-MeC27). Here again, these difference were only found in our absolute amounts analysis and not in our relative amounts analysis. We interpret these findings as an indication of absolute amount carrying additional information involved in sexual selection, while not masking information carried by relative amounts.

Two main issues can be addressed in light of our results:

First, although bioassays are needed in order to demonstrate active sex pheromones, the significant differences between gynes and drones suggest that CHCs carry signals related to mating. These chemical cues may play different roles such as altering acceptance threshold, acting as reliable signals of fitness and fertility or kinship. Our results also show that there is an abundance of branched hydrocarbons in the CHC coating of both gynes and drones and it is known that such compounds reduce the waterproofing efficacy of the cuticle (Gibbs and Pomonis 1995). This reduced efficiency may, therefore, also play a role in signaling fitness, through the handicap principle (Heinze and d'Ettorre 2009; Zahavi and Zahavi 1999) as it has been suggested before (Boulay *et al.* 2017).

Second, we have shown that it is important to analyze both total amounts and relative amounts. Our study showed them both to be significantly different between gynes and drones. Absolute and relative amounts may carry distinct cues; Absolute quantities may be indicative of body size, carrying signals of overall fitness and fertility, while relative amount may be cues for kinship. Therefore, it is not unlikely that both absolute quantities and relative amounts influence mate choice and mating behavior and that both drones and gynes integrate multiple cues. We encourage future studies to account for both factors and hope that future bio-assays will help identify specific sex pheromones as well as other signals affecting sexual behavior and mating in the *Cataglyphis* genus.

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REFERENCES

Abell AJ, Cole BJ, Reyes R, Wiernasz DC (1999) Sexual selection on body size and shape in the western harvester ant, Pogonomyrmex occidentalis Cresson. Evolution 53: 535-545

Antonialli Junior W, Lima S, Andrade L, Súarez Y (2007) Comparative study of the cuticular hydrocarbon in queens, workers and males of Ectatomma vizottoi (Hymenoptera, Formicidae) by Fourier transform-infrared photoacoustic spectroscopy. Genetics and Molecular Research 6: 492-499

Ayasse M, Paxton R, Tengö J (2001) Mating behavior and chemical communication in the order Hymenoptera. Annual review of entomology 46: 31-78

BENTO JMS, DELLA LUCIA TM, Do Nascimento RR, Bergmann J, Morgan ED (2007) Response of workers of Atta sexdens rubropilosa (Hymenoptera: Formicidae) to mandibular gland compounds of virgin males and females. Physiological entomology 32: 283-286

Boomsma J (1991) Adaptive colony sex ratios in primitively eusocial bees. Trends in Ecology & Evolution 6: 92-95

Boomsma J, Grafen A (1990) Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. Evolution 44: 1026-1034

Boomsma J, Grafen A (1991) Colony-level sex ratio selection in the eusocial Hymenoptera. Journal of evolutionary Biology 4: 383-407

Boomsma JJ, Baer B, Heinze J (2005) The evolution of male traits in social insects. Annu Rev Entomol 50: 395-420

Boulay R, Aron S, Cerdá X, Doums C, Graham P, Hefetz A, Monnin T (2017) Social life in arid environments: the case study of Cataglyphis ants. Annual review of entomology 62: 305-321

Bourke AF (2005) Genetics, relatedness and social behaviour in insect societies. SYMPOSIUM-ROYAL ENTOMOLOGICAL SOCIETY OF LONDON. p. 1

Brand J, Duffield R, MacConnell J, Blum M, Fales H (1973a) Caste-specific compounds in male carpenter ants. Science 179: 388-389

Brand J, Fales H, Sokoloski E, MacConnell J, Blum M, Duffield R (1973b) Identification of mellein in the mandibular gland secretions of carpenter ants. Life sciences 13: 201-211

Brodetzki TR, Inbar S, Cohen P, Aron S, Privman E, Hefetz A (2019) the Interplay between Incipient species and social polymorphism in the Desert Ant Cataglyphis. Scientific reports 9: 9495

Campos MCG, Campos MLG, Turatti IC, Nascimento FS (2012) Cuticular hydrocarbon variation of castes and sex in the weaver ant Camponotus textor (Hymenoptera: Formicidae). Sociobiology 59: 1025

Carlin NF (1988) Discrimination between and within colonies of social insects: two null hypotheses. Netherlands Journal of Zoology 39: 86-100

Castracani C, Tamarri V, Grasso D, Le Moli F, Palla G, Millar J, Francke W, Mori A (2008) Chemical communication in mating behaviour of the slave-making ant Polyergus rufescens (Hymenoptera, Formicidae): 3-ethyl-4-methylpentanol as a critical component of the queen sex pheromone. Insectes sociaux 55: 137-143

Castracani C, Visicchio R, Grasso DA, Mori A, Le Moli F, Di Tullio A, Reale S, De Angelis F (2005) Behavioral bioassays testing the methyl 6-methylsalicylate as a component of the female sex pheromone in the slave-making ant Polyergus rufescens (Hymenoptera, Formicidae). Journal of insect behavior 18: 685-692

Chan GL, Bourke AF (1994) Split sex ratios in a multiple-queen ant population. Proc R Soc Lond B 258: 261-266

Chapuisat M, Keller L (1999) Testing kin selection with sex allocation data in eusocial Hymenoptera. Heredity 82: 473

Chernenko A, Holman L, Helanterä H, Sundström L (2012) Cuticular chemistry of males and females in the ant Formica fusca. Journal of chemical ecology 38: 1474-1482

Coyne JA (1996) Genetics of a difference in male cuticular hydrocarbons between two sibling species, Drosophila simulans and D. sechellia. Genetics 143: 1689-1698

Cremer S, Sledge MF, Heinze J (2002) Chemical mimicry: male ants disguised by the queen's bouquet. Nature 419: 897

Cuvillier-Hot V, Cobb M, Malosse C, Peeters C (2001) Sex, age and ovarian activity affect cuticular hydrocarbons in Diacamma ceylonense, a queenless ant. Journal of Insect Physiology 47: 485-493

Davidson DW (1982) Sexual selection in harvester ants (Hymenoptera: Formicidae: Pogonomyrmex). Behavioral Ecology and Sociobiology 10: 245-250

Dietemann V, Peeters C, Liebig J, Thivet V, Hölldobler B (2003) Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant Myrmecia gulosa. Proceedings of the National Academy of Sciences 100: 10341-10346

Eyer P, Seltzer R, Reiner-Brodetzki T, Hefetz A (2017) An integrative approach to untangling species delimitation in the Cataglyphis bicolor desert ant complex in Israel. Molecular phylogenetics and evolution 115: 128-139

Franks NR, Hölldobler B (1987) Sexual competition during colony reproduction in army ants. Biological Journal of the Linnean Society 30: 229-243

Gibbs A, Pomonis JG (1995) Physical properties of insect cuticular hydrocarbons: the effects of chain length, methyl-branching and unsaturation. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 112: 243-249

Grafen A (1986) Split sex ratios and the evolutionary origins of eusociality. Journal of Theoretical Biology 122: 95-121

Grafen A (1990) Do animals really recognize kin? Animal Behaviour 39: 42-54

Grasso DA, Visicchio R, Castracani C, Mori A, Le Moli F (2003) The mandibular glands as a source of sexual pheromones in virgin queens of Polyergus rufescens (Hymenoptera, Formicidae). Italian Journal of Zoology 70: 229-232

Greenberg L, Johnson CA, Trager JC, McElfresh JS, Rodstein J, Millar JG (2018) Sex Attractant Pheromones of Virgin Queens of Sympatric Slave-Making Ant Species in the Genus Polyergus, and their Possible Roles in Reproductive Isolation. Journal of chemical ecology: 1-9

Greenberg L, Tröger AG, Francke W, McElfresh JS, Topoff H, Aliabadi A, Millar JG (2007) Queen sex pheromone of the slave-making ant, Polyergus breviceps. Journal of chemical ecology 33: 935-945

Greene MJ, Gordon DM (2003) Social insects: cuticular hydrocarbons inform task decisions. Nature 423: 32

Grillet M, Dartevelle L, Ferveur J-F (2005) A Drosophila male pheromone affects female sexual receptivity. Proceedings of the Royal Society B: Biological Sciences 273: 315-323

Heinze J, d'Ettorre P (2009) Honest and dishonest communication in social Hymenoptera. Journal of Experimental Biology 212: 1775-1779

Heinze J, Hölldobler B, Yamauchi K (1998) Male competition in Cardiocondyla ants. Behavioral ecology and sociobiology 42: 239-246

Helft F, Doums C, Monnin T (2016) No evidence of pre-copulatory mate choice by gynes in the facultatively parthenogenetic ant Cataglyphis cursor. Insectes sociaux 63: 199-201

Helft F, Monnin T, Doums C (2015) First evidence of inclusive sexual selection in the ant Cataglyphis cursor: worker aggressions differentially affect male access to virgin queens. Ethology 121: 641-650

Herbers JM (1984) Queen-worker conflict and eusocial evolution in a polygynous ant species. Evolution 38: 631-643

Hojo MK, Wada-Katsumata A, Akino T, Yamaguchi S, Ozaki M, Yamaoka R (2008) Chemical disguise as particular caste of host ants in the ant inquiline parasite Niphanda fusca (Lepidoptera: Lycaenidae). Proceedings of the Royal Society B: Biological Sciences 276: 551-558

Hölldobler B (1976) The behavioral ecology of mating in harvester ants (Hymenoptera: Formicidae: Pogonomyrmex). Behavioral Ecology and Sociobiology 1: 405-423

Hölldobler B, Engel-Siegel H (1984) On the metapleural gland of ants. Psyche: A Journal of Entomology 91: 201-224

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press,

Johnson CA, Sundström L (2012) Cuticular chemistry of two social forms in a facultatively polygyne ant (Hymenoptera: Formicidae: Formica truncorum). Annales Zoologici Fennici. JSTOR, pp. 1-17
Keller L, Aron S, Passera L (1996) Internest sex-ratio variation and male brood survival in the ant Pheidole pallidula. Behavioral Ecology 7: 292-298
Keller L, Passera L (1993) Incest avoidance, fluctuating asymmetry, and the consequences of inbreeding in Iridomyrmex humilis, an ant with multiple queen colonies. Behavioral Ecology and Sociobiology 33: 191-199
Kinomura K, Yamauchi K (1987) Fighting and mating behaviors of dimorphic males in the ant. Journal of Ethology 5: 75-81
Kureck IM, Neumann A, Foitzik S (2011) Wingless ant males adjust mate-guarding behaviour to the competitive situation in the nest. Animal behaviour 82: 339-346
Lahav S, Soroker V, Hefetz A, Vander Meer RK (1999) Direct behavioral evidence for hydrocarbons as ant recognition discriminators. Naturwissenschaften 86: 246-249
Law JH, Wilson EO, McCloskey JA (1965) Biochemical polymorphism in ants. Science 149: 544-545
Martin S, Drijfhout F (2009) A review of ant cuticular hydrocarbons. Journal of chemical ecology 35: 1151
Mehdiabadi NJ, Reeve HK, Mueller UG (2003) Queens versus workers: sex-ratio conflict in eusocial Hymenoptera. Trends in Ecology & Evolution 18: 88-93
Monnin T, Helft F, Leroy C, d'Ettorre P, Doums C (2018) Chemical characterization of young virgin queens and mated egg-laying queens in the ant Cataglyphis cursor: random forest classification analysis for multivariate datasets. Journal of chemical ecology 44: 127-136
Moritz RF, Neumann P (2004) Differences in nestmate recognition for drones and workers in the honeybee, Apis mellifera (L.). Animal behaviour 67: 681-688
Nonacs P (1986) Sex-ratio determination within colonies of ants. Evolution 40: 199-204
Oppelt A, Spitzenpfeil N, Kroiss J, Heinze J (2008) The significance of intercolonial variation of cuticular hydrocarbons for inbreeding avoidance in ant sexuals. Animal Behaviour 76: 1029-1034
Pamilo P, Seppä P (1994) Reproductive competition and conflicts in colonies of the ant Formica sanguinea. Animal Behaviour 48: 1201-1206
Queller DC, Strassmann JE (1998) Kin selection and social insects. Bioscience 48: 165-175
Ratnieks FL, Boomsma JJ (1997) On the robustness of split sex ratio predictions in social Hymenoptera. Journal of theoretical biology 185: 423-439
Smith BH (1983) Recognition of female kin by male bees through olfactory signals. Proceedings of the national Academy of Sciences 80: 4551-4553
Stuart R, Francoeur A, Loiselle R (1987) Lethal fighting among dimorphic males of the ant, Cardiocondyla wroughtonii. Naturwissenschaften 74: 548-549
Sundström L (1994) Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. Nature 367: 266
Trivers RL, Hare H (1976) Haploidploidy and the evolution of the social insect. Science 191: 249-263
Van Oystaeyen A, Oliveira RC, Holman L, van Zweden JS, Romero C, Oi CA, d'Ettorre P, Khalesi M, Billen J, Wäckers F (2014) Conserved class of queen pheromones stops social insect workers from reproducing. Science 343: 287-290
Walter F, Fletcher D, Chautems D, Cherix D, Keller L, Francke W, Fortelius W, Rosengren R, Vargo E (1993) Identification of the sex pheromone of an ant, Formica lugubris (Hymenoptera, Formicidae). Naturwissenschaften 80: 30-34
Wiernasz DC, Sater AK, Abell AJ, Cole BJ (2001) Male size, sperm transfer, and colony fitness in the western harvester ant, Pogonomyrmex occidentals. Evolution 55: 324-329
Wiernasz DC, Yencharis J, Cole BJ (1995) Size and mating success in males of the western harvester ant, Pogonomyrmex occidentalis (Hymenoptera: Formicidae). Journal of insect behavior 8: 523-531
Zahavi A, Zahavi A (1999) The handicap principle: A missing piece of Darwin's puzzle. Oxford University Press,