bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

SHANI INBAR[*], EYAL PRIVMAN

Department of Evolutionary and Environmental Biology, Institute of Evolution, University of Haifa, Haifa 43498838, Israel

[*] Corresponding author: shaniinbar@gmail.com ORCID identifier 0000-0002-1537-4393

Abstract-

Cuticualar 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 species that exhibits split sex-ratio and found significant quantitative differences between virgin queens and their potential mates. In an analyses of both absolute amounts and relative amounts, 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, Sexual selection, Sex-specific cuticular hydrocarbons.

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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, 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, however, 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 (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 males (Grasso et al. 2003). In several species, males 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

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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) and 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 males able to get closer to gynes without being harassed by workers (Helft et al. 2016; Helft et al. 2015). There is, indeed, evidence of males mimicking the queen’s chemical bouquet in ants, thus allowing them to escape aggression from other males (Cremer et al. 2002) or from workers (Franks and Hölldobler 1987). Other such acceptance-

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

altering effects have been demonstrated in honeybees where different nestmate recognition mechanisms are used to identify drones and workers (Moritz and Neumann 2004) enabling males to immigrate to foreign colonies.

Kinship between gynes and males 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 males. Body size, in both sexes, is a reliable indicator of fitness and, in Pogonomyrmex harvester ants, for example, larger males 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 influence the absolute amounts of CHCs on an individual ant and the total amount of a component of the cuticular lipid 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

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

with other chemicals, or both factors combined. Ratios between CHCs are usually less variable than are the absolute quantities of each hydrocarbon (See for example in Drosophila Coyne (1996)) because ratios are insensitive to 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 in both gynes and drones may play a role in mating. We, therefore, chose a twofold analysis of the CHCs to account for the possibility of absolute amounts enhancing differences between gynes and males in ants of the *C. niger* species complex 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

Gynes and males were collected on the spring of 2016 from colonies dug in Betzet beach on the northern Israeli coastline (from N33.05162, E35.10245 to N33.07868, E35.10705). This population was previously described as *C. drusus* (Eyer et al. 2017) but our recent species delimitation study raised the question of whether *C. drusus* is separate species or is it the same species as *C. niger*, because these populations are not differentiated by their nuclear genomic DNA (Reiner-Brodetzki et al. 2018). Colonies of this population are monogyne (headed by a single queen), polyandrous (queens are multiply mated), and monodomous (single nest per colony) (Eyer et al. 2017). We recently reported that this population exhibits split sex-ratio

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

allocation, that is, colonies produce either gynes or males but never both sexulas (Inbar and Privman 2018). Queens usually mate during the spring and sexuals can be found in nests in early spring. In the present study we used samples from twelve nests, 6 female-producing colonies and 6 male-producing colonies, with 1-3 gynes/drones collected from each colony. 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. 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 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.

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

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 methods are shown in Figure 2.

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. In a linear discriminant analysis (LDA) followed by unidimensional test of equality of the means of the groups, the two analyses showed different compounds to be significantly discriminating between gynes and drones, namely, 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).

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

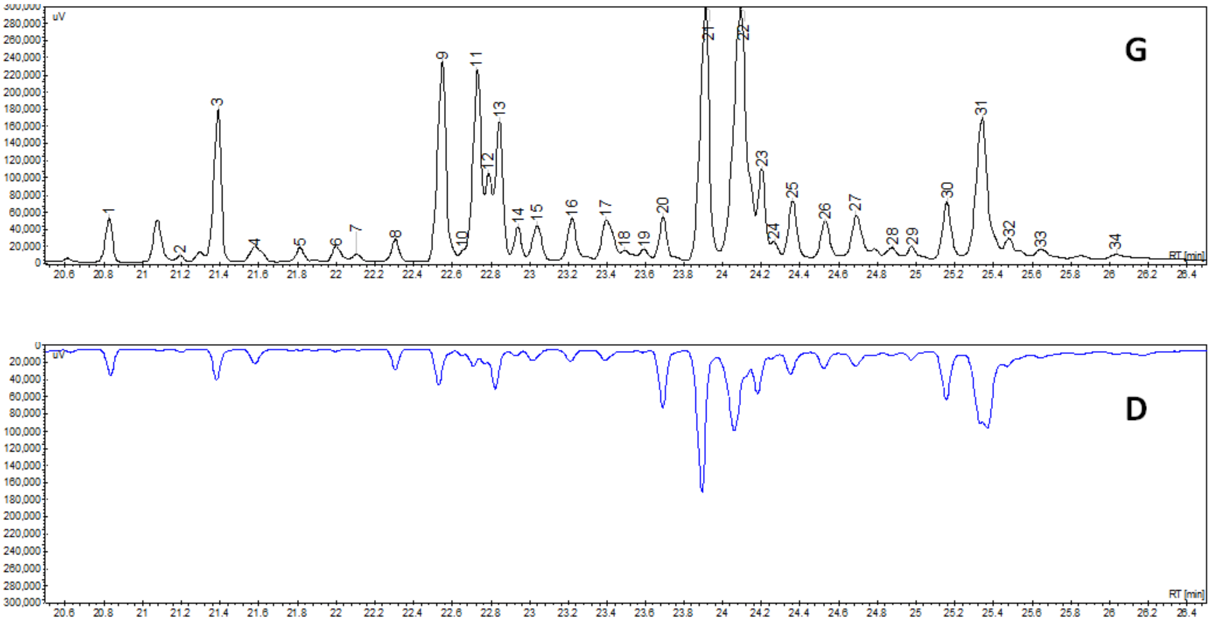
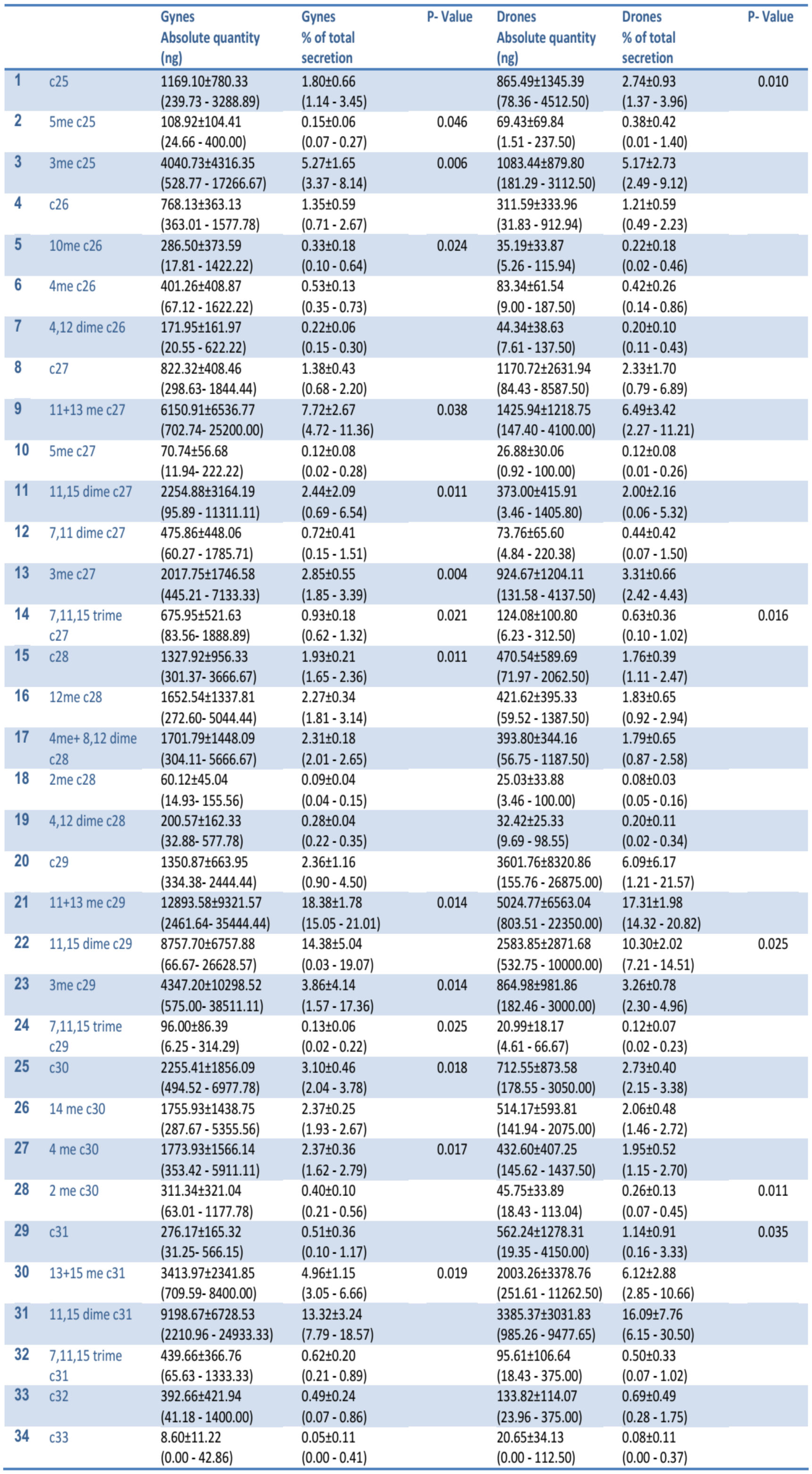


Figure 1: Chromatograms of CHCs from total body extracts. The upper chromatogram is of a gyne and the lower of a drone. Only long-chained CHC are shown.

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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



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

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

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

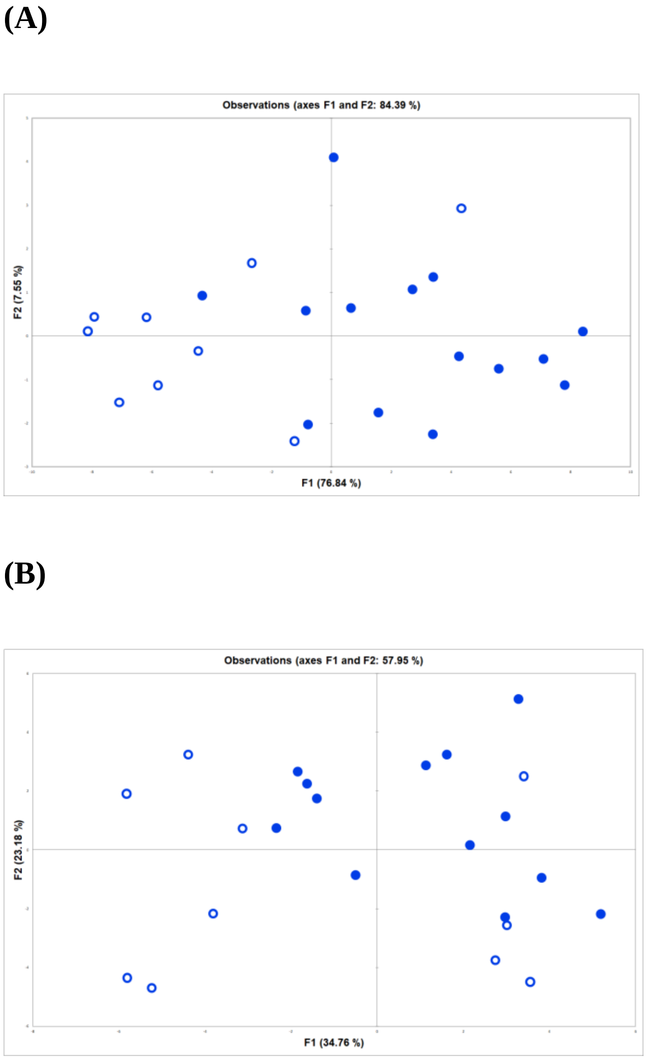


Figure 2: Principal component analysis (PCA). **(A)** Absolute quantities; **(B)** percent of total extract. Filled blue points represent individual observations of gynes and empty points of drones.

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

DISCUSSION

Our study reveals differences in long-chained CHCs between drones and gynes in a population of *Cataglyphis* colonies that display split-sex ratios. 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.

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

First, although bioassays are needed in order to demonstrate active sex pheromones, the robust 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 males and gynes integrate multiple cues. We

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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.

Acknowledgement

E.P. was supported by Israel Science Foundation Grants no. 646/15, 2140/15, and 2155/15 and US-Israel Binational Science Foundation Grant no. 2013408

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 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

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

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 et al. (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 et al. (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

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 (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

bioRxiv preprint doi: https://doi.org/10.1101/538926; this version posted February 2, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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

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,

Inbar S, Privman E (2018) Split sex-ratio and variability in aggressive behavior in the ant Cataglyphis niger bioRxiv:478552

Johnson CA, Sundström L Cuticular chemistry of two social forms in a facultatively polygyne ant (Hymenoptera: Formicidae: Formica truncorum). In: Annales Zoologici Fennici, 2012. JSTOR, pp 1-17

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

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

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

Reiner-Brodetzki T, Inbar S, Cohen P, Aron S, Privman E, Hefetz A (2018) Incipient species or social polymorphism? Diversity in the desert ant Cataglyphis bioRxiv:501270

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

Van Oystaeyen A et al. (2014) Conserved class of queen pheromones stops social insect workers from reproducing Science 343:287-290

Walter F et al. (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,