Cuticular Hydrocarbon Variation of Castes and Sex in the Weaver Ant *Camponotus textor* (Hymenoptera: Formicidae)

by

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ABSTRACT

Cuticular hydrocarbons play important roles as chemical signatures of individuals, castes, sex and brood. They also can mediate the regulation of egg laying in ants, by informing directly or indirectly the reproductive status of queens. In this study we asked whether cuticular hydrocarbon profiles are correlated with castes and sex of *Camponotus textor*. Cuticular hydrocarbons were extracted from part of a mature colony (80 workers, 27 major workers, 27 queens, 27 virgin queens and 27 males). Results showed that cuticular hydrocarbons varied quantitatively and qualitatively among the groups and this variation was sufficiently strong to allow separation of castes and genders. We discuss the specificity of some compounds as possible regulatory compounds of worker tasks and reproduction in *C. textor*.

Key-words: cuticular hydrocarbons, *Camponotus textor*, castes and sex

INTRODUCTION

The integrity of social insect colonies is possible due to the ability of individuals to recognize and to discriminate nestmates, non-nestmates and heterospecific individuals (Wilson 1971; Hölldobler & Michener 1980; Breed & Bennett 1987; Nunes *et al.* 2008). In a nestmate recognition system, individuals detect and perceive the odor present in the cuticle of another individual and compare it with an internal template, thus distinguishing nestmate cues from non-nestmate cues (Sturgis & Gordon 2012; Leonhardt *et al.* 2007). Several studies have shown that cuticular hydrocarbons are the main compounds playing roles as chemical signatures of individuals, castes,

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brood and colonies (Vander Meer & Morel 1998; Tannure-Nascimento *et al.* 2009; reviewed in Blomquist & Bagneres 2010).

In ants, for example, workers that perform distinct tasks present variable hydrocarbon profiles (Wagner *et al.* 1998). Patrollers and foragers of *Pogonomyrmex barbatus* yield a higher amount of more complex compounds than nest maintenance workers (Wagner *et al.* 2001). In this same species, Greene and Gordon (2003) showed that a worker can access the task status of a nestmate from its cuticular hydrocarbon profile. In addition, Kaib *et al.* (2000) showed that, irrespective to their age, *Myrmicaria eumenoides* workers performing nest tasks retain their hydrocarbon profiles unaltered. They stated that the variation of the cuticular blend is not only relatively correlated with age, but also strongly correlated with task allocation of each individual.

Moreover, cuticular hydrocarbons can mediate the reproduction and regulation of egg laying in ants (Peeters *et al.* 1999; Liebig *et al.* 2000) and the reproductive status is related to the cuticular hydrocarbons of queens, mated workers and gamergates (Monnin *et al.* 1998; Liebig *et al.* 2000; Cuvillier-Hot *et al.* 2001). Reproductive queens have differences in their cuticular profiles when compared with infertile workers (Heinze *et al.* 2002). In *Camponotus floridanus* the presence and the fertility of queen may be indicated by presence of hydrocarbons directly from the queen or her eggs (Endler *et al.* 2004).

Camponotus textor is a weaver ant that inhabits forests of Central and South America (Schremmer 1979a, 1979b). Their nests are built with larval silk and present a round shape with several galleries around the branches and dead leaves (Santos 2002; Longino 2006). Behavioral observations and natural history of this species has previously been studied (cited as *C. senex*: Santos *et al.* 2005a, 2005b; Santos & Del-Claro 2009).

In the present study we evaluated and compared the chemical profile of cuticles of *C. textor* workers, queens, virgin queens and males.

MATERIAL AND METHODS

A mature colony (27 queens, approximately 24,600 workers and major workers, approximately 500 virgin queens and 500 males) of *C. textor* obtained from the University of São Paulo (USP-Ribeirão Preto, São Paulo, Brazil), was collected and freezer-killed (-20°C). The cuticular hydrocarbons of 80 workers, 27 major workers, 27 queens, 27 virgin queens and 27 males were extracted.

Cuticular compounds were extracted from individuals in 0.5 ml hexane for 2 min. After evaporating the solvent, the apolar extract was resuspended in 160 μl of hexane. The samples were analyzed in a GC-MS (Shimadzu, model QP2010 plus) equipped with silica capillary column of 30m and helium as carrier gas at 1 ml/min. The oven temperature was initially set to 150°C, and ramped up 3°C/min until it reached 280°C. Analyses were performed in splitless mode. Data were analyzed to characterize the mass spectrum. Equivalent chain lengths were determined using n-alkane standards (Sigma Chemical Co.) and quantification was based on the peak areas obtained from the chromatograms.

The data were analyzed with GCMS solutions for Windows (Shimadzu Corporation) and the chemical compounds were identified based on their mass spectra by comparison with Wiley Library data and with a standard solution of different synthetic hydrocarbons.

Principal components analysis (PCA) was used to define the main compound peaks to be compared. Compounds missing in the most individuals of an analyzed group as well as compounds contributing less than 5% to the first two factors, as indicated by the PCA, were excluded from the statistical analysis. Following this, a stepwise discriminant function analysis was used to observe if combinations of variables could be useful in the predicting group. In this method, variables are successively added to the model based on the higher F to enter values, adding no more variables when the F ratio is no longer significant. Wilks' λ values were used to verify the individual contribution of each variable to the model. The Wilks' λ statistic for the overall discrimination is computed as the ratio of the determinant of the within-groups variance/covariance matrix to the determinant of the total variance/covariance matrix. Mahalanobis distance (D) was used as parameter to evaluate chemical distance between groups.

To avoid errors in compositional sample data, the area under each peak was transformed according to the following formula: $Z = \ln[Ap/g(Ap)]$, where Ap is the area under the peak, g(Ap) is the geometric mean for each individual compound group and Z is the transformed peak area (Aitchison

1986). The statistical analyses were performed using the software Statistica 7.0 (Statsoft, Inc.).

RESULTS

Analysis of the worker cuticular waxes identified 90 hydrocarbons (Table 1). These compounds were classified as linear alkanes, monomethylalkanes, and dimethylalkanes. The groups presented many qualitative differences in their respective cuticular profiles (Table 1).

Of ninety compounds found, eighty-nine compounds were present in queens. Apart from these, eight compounds were exclusively found in the queens and six compounds were only found in queens and virgin queens. Workers, major workers and males had no exclusive compounds, but major workers and queens had four compounds in common, and one compound was only found in workers and queens.

Queens, workers and major workers presented nine compounds and these compounds were not present in virgin queens and males. One compound was present only in major workers.

Some compounds were more representative in the relative proportion of cuticular hydrocarbon profile of studied groups: 17-, 15- and 13-Me C_{32} (>10%), n-C_{28} and n-C_{30} (> 5%), 9-, 11- and 13-, 15-DiMe C_{32} (>6%), and 13-, 15-DiMe C_{29} (>4%) (Table 2).

Stepwise discriminant analyses of these compounds significantly separated castes and sex (Table 2: Global Model: Wilks' $\lambda = 0.001$; F = 52,668; $p < 0.001$) and the most important hydrocarbons for this separation (*F* to enter >1) were 9-, 11-DiMe C_{32}, 4-Me C_{30}, n-C_{32}, 13-, 15-DiMe C_{30}, n-C_{30}, n-C_{28} and n-C_{29}. Chemical distances (*D*) between castes and sex were extremely high (Fig. 1): queens vs workers: 102.57, queens vs majors: 72.92, queens vs virgin queens: 127.87, queens vs males 184.58, workers vs majors: 41.95, workers vs virgin queens: 54.16, workers vs males: 58.20, majors vs virgin queens: 98.91, majors vs males: 121.06 and virgin queens vs males: 44.85 (all values were highly significant $p<0.001$).

DISCUSSION

In social insects, physiological variation of individuals is well reflected in their cuticular hydrocarbons (reviewed in Blomquist & Bagneres 2010). In

Table 1: Cuticular hydrocarbons present in the *Camponotus textor* workers (W), major workers (LW), queens (Q), virgin queens (VQ) and males (M). RT = retention time in minutes. Different letters indicate significant differences ($p<0.001$).

COMPOUND	RT	W		LW		Q		VQ		M	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
n-C_{26}	28.841	0.54	±0.36^{a}	0.28	±0.17^{a}	0.16	±0.06^{b}	0.03	±0.03^{b}	0.10	±0.09^{b}
12-Me C_{26} 10-Me C_{26}	29.669	-		-		0.12	±0.09	0.09	±0.04	-	
3-Me C_{26}	30.661	0.40	±0.21^{a}	0.44	±0.16^{b}	0.35	±0.17^{a}	0.31	±0.05^{a}	0.14	±0.08^{c}
n-C_{27}	31.359	1.32	±1.56^{d}	0.45	±0.25^{c}	0.24	±0.11^{ac}	0.05	±0.02^{b}	0.09	±0.05^{ab}
13-Me C_{27} 11-Me C_{27} 9-Me C_{27}	32.124	0.15	±0.15	0.18	±0.05	0.15	±0.06	-		-	
5-Me C_{27}	32.725	0.12	±0.08	0.07	±0.02	0.18	±0.48	0.05	±0.02	-	
11-.13-DiMe C_{27} 13-.15-DiMe C_{27}	32.879	-		0.09	±0.03	0.16	±0.08	0.05	±0.02	0.01	±0.01
3-Me C_{27}	33.122	-		0.21	±0.09	0.11	±0.05	-		-	
n-C_{28}	33.828	9.63	±4.28^{d}	4.60	±2.68^{a}	2.75	±1.28^{ab}	0.80	±0.47^{c}	1.89	±0.75^{bc}
14-Me C_{28} 13-Me C_{28} 12-Me C_{28}	34.561	4.13	±1.72^{ac}	3.62	±0.98^{ab}	2.46	±1.20^{c}	4.26	±0.39^{b}	3.16	±0.91^{c}
7-Me C_{28}	34.745	0.43	±0.53^{b}	1.33	±0.34^{a}	0.28	±0.13^{b}	1.47	±0.17^{a}	1.02	±0.22^{a}
6-Me C_{28}	34.950	-		0.21	±0.07	0.27	±0.30	-		-	
4-Me C_{28}	35.162	0.28	±0.26^{a}	0.22	±0.06^{a}	0.19	±0.13^{a}	0.23	±0.07^{a}	0.04	±0.03^{b}
2-Me C_{28}	35.582	5.91	±1.81^{b}	5.54	±1.35^{a}	6.05	±2.76^{abc}	4.15	±0.86^{c}	8.79	±1.75^{d}
3-Me C_{28}	35.660	-		0.15	±0.06	0.06	±0.09	0.09	±0.03	-	
n-C_{29}	36.175	3.24	±1.68^{d}	1.16	±0.84^{ac}	1.78	±0.70^{c}	0.22	±0.15^{b}	0.46	±0.10^{ab}
15-Me C_{29} 13-Me C_{29} 11-Me C_{29}	36.845	0.32	±0.35^{d}	0.81	±0.41^{bc}	0.30	±0.16^{ad}	0.92	±0.15^{c}	0.54	±0.23^{ab}
9-Me C_{29}	36.970	-		0.11	±0.05	0.16	±0.11	-		-	
11-.15-DiMe C_{29}	37.454	-		0.09	±0.12	0.43	±0.85	-		-	
9-.13-DiMe C_{29}	37.593	0.21	±0.12	0.23	±0.08	0.37	±0.18	-		-	
3-Me C_{29}	37.824	0.13	±0.11^{a}	0.17	±0.07^{b}	0.38	±0.62^{b}	0.08	±0.03^{a}	0.07	±0.03^{a}
n-C_{30}	38.491	7.22	±3.11^{b}	3.91	±2.40^{a}	6.56	±2.73^{b}	1.00	±0.68^{a}	2.77	±0.95^{a}
13-. 15-DiMe C_{29}	38.941	5.12	±1.63	-		1.74	±2.34	9.29	±1.28	11.15	±2.87
16-Me C_{30} 14-Me C_{30} 12-Me C_{30}	39.111	0.58	±0.42^{ab}	7.71	±1.89^{d}	1.00	±0.86^{a}	0.30	±0.09^{bc}	0.19	±0.09^{c}

Table 1 (continued): Cuticular hydrocarbons present in the *Camponotus textor* workers (W), major workers (LW), queens (Q), virgin queens (VQ) and males (M). RT = retention time in minutes. Different letters indicate significant differences ($p<0.001$).

COMPOUND	RT	**W**		**LW**		**Q**		**VQ**		**M**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10-Me C_{30}	39.205	1.11	$\pm0.57^{d}$	0.26	$\pm0.10^{c}$	1.61	$\pm0.78^{a}$	1.96	$\pm0.25^{b}$	1.89	$\pm0.40^{ab}$
?	39.321	0.76	±0.63	1.86	±0.42	1.11	±0.39	-		-	
17-Me C_{30} 15-Me C_{30} 13-Me C_{30}	39.556	1.24	±0.52	-		0.82	±0.84	1.24	±0.13	0.21	±0.40
11-.15-DiMe C_{30}	39.683	1.17	$\pm0.56^{ab}$	0.79	$\pm0.30^{c}$	0.65	$\pm0.37^{bd}$	0.46	$\pm0.08^{acd}$	1.36	$\pm1.21^{d}$
9-. 17-Di Me C_{30}	39.756	1.29	$\pm0.69^{a}$	0.65	$\pm0.48^{ab}$	0.46	$\pm0.28^{b}$	2.72	$\pm1.42^{a}$	8.86	$\pm2.84^{c}$
13-.15 DiMe C_{30}	39.939	2.61	$\pm1.53^{a}$	1.46	$\pm0.53^{a}$	0.45	$\pm0.33^{b}$	1.71	$\pm0.80^{a}$	0.65	$\pm0.38^{b}$
4-Me C_{30}	40.151	4.04	$\pm1.23^{b}$	3.92	$\pm1.77^{b}$	7.70	$\pm3.24^{c}$	2.05	$\pm0.75^{a}$	1.04	$\pm0.19^{a}$
n-C_{31}	40.680	1.41	$\pm0.59^{d}$	0.71	$\pm0.39^{ab}$	3.82	$\pm3.26^{c}$	0.73	$\pm0.28^{b}$	0.47	$\pm0.13^{a}$
14-Me C_{31} 15-Me C_{31}	41.279	1.19	$\pm0.45^{b}$	2.15	$\pm0.68^{a}$	1.17	$\pm0.58^{b}$	2.27	$\pm0.60^{a}$	1.59	$\pm0.28^{c}$
9-Me C_{31}	41.435	-		-		0.14	±0.07	-		-	
5-Me C_{31}	41.579	-		-		0.45	±1.00	-		-	
11-.15-DiMe C_{31} 9-.13-DiMe C_{31}	41.898	0.52	$\pm0.21^{b}$	0.96	$\pm0.30^{a}$	1.50	$\pm0.90^{a}$	0.43	$\pm0.19^{b}$	0.25	$\pm0.13^{c}$
7-. 15-DiMe C_{31} 7-. 17-DiMe C_{31}	41.998	-		-		0.59	±0.41	-		-	
5-. 13-DiMe $C_{31.}$ 5-. 15-DiMe C_{31} 5-. 17-DiMe C_{31}	42.282	-		0.36	±0.14	0.52	±0.24	0.58	±0.20	-	
3-Me C_{31}	42.465	-		-		0.39	±0.41	0.12	±0.05	-	
n-C_{32}	42.843	1.47	$\pm0.79^{a}$	0.74	$\pm0.45^{a}$	5.36	$\pm2.06^{c}$	0.16	$\pm0.13^{b}$	0.33	$\pm0.11^{b}$
13-Me C_{32} 15-Me C_{32} 17-Me C_{32}	43.415	13.76	$\pm3.67^{d}$	12.73	$\pm2.99^{a}$	7.14	$\pm3.72^{b}$	15.21	$\pm1.02^{c}$	15.12	$\pm2.48^{a}$
9-Me C_{32}	43.533	1.95	$\pm0.50^{a}$	0.37	$\pm0.33^{c}$	2.84	$\pm1.10^{b}$	2.04	$\pm0.19^{b}$	2.24	$\pm0.74^{ab}$
7-Me C_{32}	43.645	-		2.41	±0.56	2.02	±0.71	-		-	
10-. 14-DiMe C_{32} 5- Me C_{32}	43.859	5.00	$\pm1.55^{c}$	2.00	$\pm0.66^{a}$	4.46	$\pm2.61^{bc}$	2.87	$\pm0.32^{ab}$	4.53	$\pm2.35^{bc}$
12- 16-DiMe C_{32}	43.957	1.12	$\pm0.60^{c}$	1.98	$\pm0.60^{a}$	2.00	$\pm2.24^{bc}$	1.27	$\pm0.18^{ab}$	3.17	$\pm1.99^{a}$
9-. 11-DiMe C_{32} 13-.15-DiMe C_{32}	44.055	4.15	$\pm1.12^{c}$	0.95	$\pm0.29^{b}$	1.54	$\pm1.11^{b}$	9.45	$\pm0.99^{a}$	7.27	$\pm1.42^{a}$
6-. 10-DiMe C_{32} 6-. 14-DiMe C_{32} 6-. 16-DiMe C_{32}	44.222	2.52	±1.40	7.66	±2.08	1.50	±0.79	-		-	

Table 1 (continued): Cuticular hydrocarbons present in the *Camponotus textor* workers (W), major workers (LW), queens (Q), virgin queens (VQ) and males (M). RT = retention time in minutes. Different letters indicate significant differences (p<0.001).

COMPOUND	RT	W		LW		Q		VQ		M	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
n- C_{33}	44.519	2.28	±0.61^{c}	1.30	±0.34^{ab}	10.91	±4.25^{d}	1.62	±0.23^{bc}	0.91	±0.28^{a}
13-Me $C_{33.}$ 15-Me C_{33} 17-Me C_{33}	45.015	-		-		1.23	±0.54	1.54	±0.22	-	
9-Me C_{33}	45.153	0.06	±0.10	-		0.78	±0.49	0.78	±0.19	-	
7-Me C_{33}	45.684	0.64	±0.51	0.31	±0.12	0.93	±0.48	-		-	
9-. 11-Di Me C_{33} 9-.13-DiMe C_{33}	45.759	-		-		0.59	±0.38	-		-	
6-Me C_{33}	46.070	0.10	±0.24	-		0.29	±0.19	3.93	±0.76	-	
5-Me C_{33}	46.300	0.21	±0.26	1.22	±0.41	0.53	±0.74	-		-	
3-Me C_{33}	46.426	-		-		0.93	±1.62	-		-	
n-C_{34}	47.648	-		-		0.96	±0.90	3.70	±0.42	-	
13-Me C_{34}	48.531	2.41	±2.23^{ad}	3.04	±0.97^{d}	2.62	±2.36^{b}	3.97	±0.48^{abc}	2.86	±0.55^{c}
11-Me C_{34}	48.620	1.62	±1.55^{c}	2.51	±0.74^{b}	1.09	±1.13^{c}	3.53	±0.73^{a}	3.11	±0.93^{ab}
5-Me C_{34}	49.174	1.76	±0.64	-		0.89	±0.70	2.95	±0.54	1.95	±0.43
7-. 11-DiMe C_{34}	49.340	-		1.76	±0.67	-		-		-	
9-. 11-DiMe C_{34}	49.413	2.00	±0.74^{b}	0.84	±0.27^{a}	1.28	±0.65^{a}	1.54	±0.46^{b}	1.11	±0.34^{a}
5-. 7-DiMe C_{34}	49.635	-		1.19	±0.42	1.12	±0.57	-		-	
n-C_{35}	49.971	0.12	±0.17	-		3.41	±1.68	-		-	
8-Me C_{35}	51.046	-		-		1.29	±0.76	-		-	

Table 2: Classification matrix for group comparison of predicted functional group using discriminant analysis.

	% Correct	W	LW	Q	VQ	M
W	98.76	80	1	0	0	0
LW	100	0	27	0	0	0
Q	100	0	0	27	0	0
VQ	88.88	0	0	0	24	3
M	100	0	0	0	0	27
Total	97.88	80	28	27	24	30

this study, castes and sex had qualitative differences in their respective cuticular profiles, mainly related to monomethylalkanes, and dimethylalkanes.

The compounds that have distinct and variable isomers (double bonds or methyl groups) are those associated with colonial recognition. On the other hand, *n*-alkanes are considered important for physical protection of the individual (Howard & Blomquist 2005).

In *C. textor*, some compounds were only found in queens and virgin queens, which suggest that these hydrocarbons are characteristics of reproductive females. In *Camponotus floridanus*, the prime distinction of cuticular hydrocarbon profiles is observed between queens and workers, in which eighteen compounds are only found in fertile queens (Endler *et al.* 2004, 2006). In *Harpegnathos saltator* the hydrocarbon profiles were similar between gamergates and queens, but they were different between workers and young queens (Peeters & Hölldobler 1995), and in *Pachycondyla inversa* many compounds were different in profiles of queens and workers (Heinze *et al.* 2002). In the polygynous ant *Linepithema humile* the cuticular hydrocarbon profiles of queens, young queens and workers were different (de Biseau *et al.* 2004). Dietemann *et al.* (2003) showed that *Myrmecia gulosa* had differences between queen and worker hydrocarbon profiles. Dahbi & Lenoir (1998) showed the similarity of chemical compound profiles in queens and their

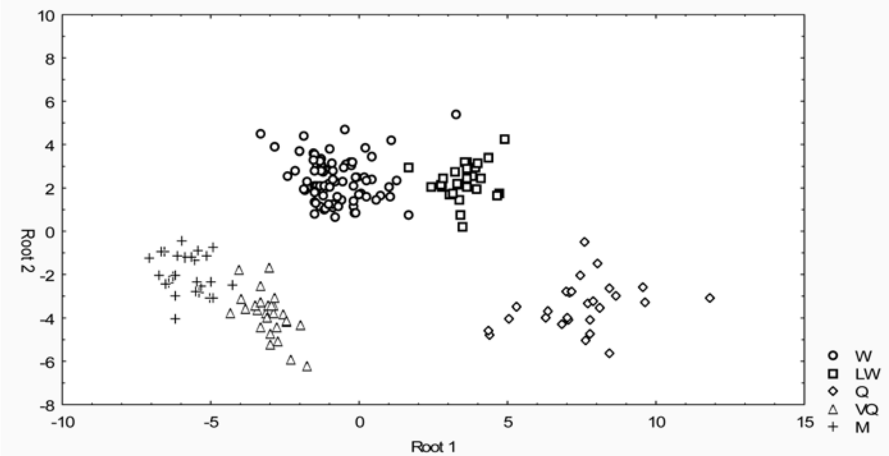

Fig. 1: Discriminant analysis of cuticular hydrocarbons present in the castes. W (workers), LW (major workers), Q (queens), VQ (Virgin queens) and M (males).

respective daughter-workers, indicating a significant correlation between cuticular profiles and fertility.

In *C. textor*, no alkenes were present in the cuticular profiles. The same results were obtained for *C. floridanus* (Endler *et al.* 2004) and *C. fellah* (Lalzar *et al.* 2010).This pattern is different from that found in *Pogonomyrmex barbatus* (Wagner *et al.* 1998), *Harpegnathos saltator* (Liebig *et al.* 2000) and other 37 ant species (van Wilgenburg *et al.* 2011).

Discriminant analysis of compounds of all studied groups showed that of 90 total compounds, 42 were common to all castes (queens and workers) and genders (females and males). 4-Me C_{30}, n-C_{32} and n-C_{33} were found in larger quantities in queens than in other groups, major workers had the compounds 16-,14- and 12-Me C_{30} in more representative quantities than any other group. Males had the compound 9-, 17-DiMe C_{30} in larger quantities than other groups and virgin queens and males presented the compound 9-, 11- and 13-, 15-DiMe C_{32} in larger quantities than other groups. Boulay *et al.* (2007) observed quantitative differences in the profile of *Aphaenogaster senile*, where reproductive queens had five compounds in larger quantities and workers had five other compounds in larger quantities. Moreover, Endler *et al.* (2006) showed that queens had a higher proportion of compounds compared with workers. Also in *Crematogaster smithii*, workers, virgin queens, intermorphs and queens had qualitative differences in their compounds (Oettler *et al.* 2008).

Colonial blend or Gestalt colony odor seems to be more important for individual recognition than a specific group of hydrocarbons. However, recent revision of cuticular hydrocarbon profiles in ants suggests that dimethylalkanes are good candidates for species and nestmate discrimination (reviewed in Martin & Drijfhout 2009a). In *C. textor*, ten of ninety compounds were dimethylalkanes, and discriminant analysis showed that variation between groups was mostly yielded by these hydrocarbons. However, further behavioral bioassays are needed to understand the role of these compounds within the colonies.

In summary, our study demonstrates that cuticular hydrocarbons varied sufficiently among castes and sex to separate individuals according to their functional roles. By using this variation, individuals may discriminate among workers, major workers, fertile queens, virgin queens and males like in other ant species. Variation in cuticular hydrocarbons might therefore play an important role in the organization of colonies of polygynous ant species.

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REFERENCES

Aitchison, J. 1986. The statistical analysis of compositional data, monographs on statistics and Applied Probability. Chapman and Hall, London.

Blomquist, G.J. & A.G Bagnères 2010. Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology. Cambridge University Press: 492.

Boulay, R., X. Cerdá, T. Simon, M. Roldan, & A. Hefetz 2007. Intraspecific competition in the ant *Camponotus cruentatus*: should we expect the 'dear enemy' effect? Animal Behaviour 1: 1-9.

Breed, M.D. & B. Bennett 1987. Kin recognition in highly eusocial insects. In: Fletcher, D J.C. & Michener, C.D. (Eds). Kin Recognition in Animals. New York: J. Wiley: 243-285.

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

Dahbi, A. & A. Lenoir 1998. Queen and colony odour in the multiple nest ant species, *Cataglyphis iberica* (Hymenoptera, Formicidae). Insectes sociaux 45: 301–313.

de Biseau, J.C., L. Passera, D. Daloze & S. Aron 2004. Ovarian activity correlates with extreme changes in cuticular hydrocarbon profile in the highly polygynous ant, *Linepithema humile*. Journal Insect Physiology 50: 585–593.

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

Endler, A., J. Liebig, T. Schmitt, J.E. Parker, G.R. Jones, P. Schreier & B. Hölldobler 2004. Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. Proceedings of the National Academy of Sciences of the United States of America 101:2045-2950.

Endler, A., J Liebig & B. Hölldobler 2006. Queen Fertility, Egg Marking and Colony Size in the Ant *Camponotus floridanus*. Behavioral Ecology and Sociobiology 59: 490-499.

Greene, M.J. & D.M. Gordon 2003. Cuticular hydrocarbons inform task decisions. Nature 423: 32.

Heinze, J., B. Stengl & M.F Sledge 2002. Worker rank, reproductive status and cuticular hydrocarbon signature in the ant, *Pachycondyla* cf. *inversa*. Behavioral Ecology and Sociobiology 52:59–65.

Hölldobler, B. & C.D. Michener 1980. Mechanisms of identification and discrimination in social hymenoptera. In: Markl, H. (ed.) Evolution of Social Behaviour. Weinheim. Verlag Chemie: 35e58.

Howard, R.W., & G.J. Blomquist 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annual Review of Entomology 50: 371-93.

Kaib, M., B. Eisermann, E. Schoeters, J. Billen, S. Franke & W. Francke 2000. Task-related variation of postpharyngeal and cuticular hydrocarbon compositions in the ant *Myrmicaria eumenoides*. Journal of Comparative Physiology 186: 939-948.

Lalzar, I, T. Simon & R.K. Vander-Meer 2010. Alteration of cuticular hydrocarbon composition affects heterospecific nestmate recognition in the carpenter ant Camponotus fellah. Chemoecology 20:19–24.

Leonhardt, S.D., A.S. Brandstaetter & C.J. Kleineidam 2007. Reformation process of the neuronal template for nestmate-recognition cues in the carpenter ant *Camponotus foridanus*. Journal of Comparative Physiology 193: 993–1000.

Liebig, J., C. Peeters, N.J. Oldham, C. Markstädter & B. Hölldobler 2000. Are variations in cuticular hydrocarbons of queens and workers a reliable signal of fertility in the ant Harpegnathos saltator? Proceedings of the National Academy of Sciences of the United States of America 97: 4124–4131.

Longino, J.T. 2006. New species and nomenclatural changes for the Costa Rican ant fauna (Hymenoptera: Formicidae). Myrmecologische Nachrichten 8: 131-143.

Martin, S. & F. Drijfhout 2009a. A review of ant cuticular hydrocarbons. Journal of. Chemical Ecology 35: 1151–1161.

Monnin, T.; C. Malosse, & C. Peeters 1998. Solid-phase microextraction and cuticular hydrocarbon differences related to reproductive activity in queenless ant *Dinoponera quadriceps*. Journal of Chemical Ecology 24: 473-490.

Nunes, T.M., F.S. Nascimento, I.C. Turatti, N.P. Lopes & R. Zucchi 2008. Nestmate recognition in a stingless bee: does the similarity of chemical cues determine guard acceptance? Animal Behaviour 75: 1165-1171.

Oettler, J., T. Schmitt, G. Herzner & J. Heinze 2008. Chemical profiles of mated and virgin queens, egg-laying intermorphs and workers of the ant *Crematogaster smithi*. Journal of Insect Physiology 54: 672–679.

Peeters, C. & B. Hölldobler 1995. Reproductive cooperation between queens and their mated workers: the complex life history of an ant with a valuable nest. Proceedings of the National Academy of Sciences of the United States of America 92: 10977– 10979.

Peeters, C., T. Monnin, & C. Malosse 1999. Cuticular hydrocarbons correlated with reproductive status in a queenless ant. Proceedings of the Royal Society 266: 1323-1327.

Santos, J.C. & K. Del-Claro 2009. Ecology and behaviour of the Weaver ant *Camponotus* (*Myrmobrachys*) *senex*. Journal of Natural History 43: 1423–1435.

Santos, J.C., M. Yamamoto, F.R. Oliveira & K. Del-Claro 2005a. Behavioral Repertory of the Weaver Ant *Camponotus (Myrmobrachys) senex* (Hymenoptera: Formicidae). Sociobiology 45: 1-11.

Santos, J. C., A.P. Korndörfer & K. Del-Claro 2005b. Defensive Behavior of the Weaver Ant *Camponotus (Myrmobrachys) senex* (Formicidae: Formicinae): Drumming and Mimicry. Sociobiology 46: 1-10.

Schremmer, F. 1979a. Das Nest der neotropischen Weberameise *Camponotus (Myrmobrachys) senex* Smith (Hymenoptera: Formicidae). Zoologische Anzeiger 203: 273-282.

Schremmer, F. 1979b. Die nahezu unbekannte neotropische Weberameise *Camponotus (Myrmobrachys) senex* (Hymenoptera: Formicidae). Entomologia Generalis 5: 363-378.

Sturgis, S.J. & D.M. Gordon 2012. Nestmate recognition in ants (Hymenoptera: Formicidae): a review. Myrmecological News 16: 101-110.

Tannure-Nascimento, I. C., F.S Nascimento, J.O. Dantas & R. Zucchi 2009. Decision rules for egg recognition are related to functional roles and chemical cues in the queenless ant *Dinoponera quadriceps*. Naturwissenschaften 96: 857-861.

van Wilgenburg, E.; M.R.E. Symonds & M.A. Elgar 2011. Evolution of cuticular hydrocarbon diversity in ants. Journal of Evolutionary Biology 24: 1188–1198.

Vander Meer, R.K. & L. Morel 1998. Nestmate recognition in ants. In: Vander Meer, R.K., Breed, M.D., Espelie, K.E. & Winston, M.L. (eds) Pheromone communication in social insects: Ants, wasps, bees and termites. Westview, Boulder: 79–103.

Wagner D., M.J.F. Brown, P. Broun, W. Cuevas, L.E. Moses, D.L. Chao & D.M. Gordon 1998. Task-related differences in the cuticular hydrocarbon composition of harvester ants, *Pogonomyrmex barbatus.* Journal of Chemical Ecology 24:2021-2037.

Wagner, D., M. Tissot & D.M. Gordon 2001. Task-related environment alters the cuticular hydrocarbon composition of harvester ants. Journal of Chemical Ecology 27: 1805–1819.

Wilson, E.O. 1971. Social Insects. Science, New Series 172: 406.