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Colony and Caste Specific Cuticular Hydrocarbon Profiles in the Common Japanese Hornet, *Vespa analis* (Hymenoptera, Vespidae)

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Abstract

In this study, the influence of individual cuticular hydrocarbons on nestmate recognition in Japanese hornets was investigated. Cuticular hydrocarbons were extracted from the individuals of several colonies of Japanese hornets, *Vespa analis* Fablicius (Hymenoptera: Vespidae), and analyzed by combined gas chromatography and mass spectrometry (GC-MS). In the case of *V. analis,* the results of the GC-MS analyses showed 12 major components and 20 minor components. The results showed some intraspecific variation in the quantities of each component of the profiles even within the same colony and same cast. The number of cuticular hydrocarbon compounds of *V. analis* was relatively high compared with that of *V. crabro.* Discriminant analysis of the cuticular hydrocarbon profiles of the workers and queens showed that the wasps could be grouped by colony and by caste even if the colonies were located at a close distance. The cuticular hydrocarbon profiles of workers and males were different.

Discipline: Insect pest
Additional key words: Asian vespines, GC-MS analysis, nestmates recognition

Introduction

The Vespid hornet, which is a social insect, is a serious pest especially for people involved in field and forest operations and forestry employees. Human death by hornet attack is more frequent than death by bear or venomous snake attack. Because the hornet attacks the honeybee, it is also a major pest for farmers that use the Apis mellifera for pollination. To understand the active range of the hornet and to use this understanding for hornet control, research into vespine social behavior has been promoted. A method of controlling the hornet that does not use insecticide is necessary from an environmental protection point of view, and an effective means of controlling the hornet may lie in an investigation of its chemical ecology, including semiochemical communications, such as nestmate recognition.

Discrimination between nestmates and non-nestmates, which are usually kin and non-kin, respectively, is often represented as a higher tolerance towards nestmates than to non-nestmates, and is widely observed among social wasps[8,9,19]. Cuticular hydrocarbons have been thought to be responsible for nestmate recognition because some social wasps are known to have colony-specific hydrocarbon profiles, for example, *Dolichovespula maculate*[2], *Vespula squamosa*, *V. macurifrons*[1], *Vespa crabro*[1,3,16], *Polistes fuscatus*[7], *Polistes exclamans*[18], *Polistes biglumis bimaculatus*:[11,12], *Polistes dominulus*[4], and *Polistes satan*[21]. In the European hornet, *V. crabro*, experiments have shown that hornet workers are able to recognize the manipulation of cuticular hydrocarbon profiles[17].

If nestmate recognition based on hydrocarbon profiles is universal among social wasps, we should expect colony-specific profiles in other species irrespective of their biological or geographical peculiarities. Only a very small proportion of the vespine species, however, has ever been examined for cuticular hydrocarbons and intercolonial differences. In particular, no Asian vespines have been studied for cuticular hydrocarbon profiles.

Previous studies have shown not only that hydrocarbon components and their proportions vary among species but that the main components responsible for intercolonial differences differ between taxa as revealed by discriminant analyses[2,3,19,17].

For these reasons, it is interesting to investigate the

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components of cuticular hydrocarbons and their intercolonial differences in various taxa of social wasps. In this report, we characterize the cuticular hydrocarbons of the workers, queens, and males of the Asian hornet, *Vespa analis*, collected from the same town, and show that wasps from the same colony have similar cuticular hydrocarbon profiles and that these profiles are colony-specific and cast-specific by using discriminant analysis.

Materials and methods

1. Wasps

Vespa analis is widely distributed in southeast and east Asia including Japan, almost always nesting in open spaces[14]. Nesting is annual and haplometrotic. In Japan, nests are initiated in May and produce their first workers in June[14]. Mature nests contain 300 to 600 cells and less than 100 workers, and produce 50 to 200 reproductives of both sexes by the end of colony[14]. Although relatively smaller in colony size and less aggressive in behavior compared with many congeners, *V. analis* has become a major pest in some residential areas in Japan[13]. This is chiefly because it frequently nests in hedges or under the eaves of houses, thus attracting human attention.

2. Nest collection and sample preparation

Six colonies of *V. analis* were collected in Kukizaki (140E, 35N), Tsukuba, Ibaraki Prefecture, Japan, from August to September 2001. The colonies were found in an area of about 6 x 3 km, with the nearest adjacent nests about 500 m apart. All nests were located on houses or in hedges. We anesthetized the wasps in the nests with ether after blocking the nest entrance with a cotton plug. The collected nests were quickly brought back to the laboratory and dissected to record the nest's contents. All nests contained the founding queen together with 26 to 95 workers, and a single nest had adult males. New queens had not yet emerged in any of the nests. The adults in the nests were individually packed in small plastic bags and stored in a freezer at -20°C. Twenty workers were randomly selected from frozen samples of each colony for GC-MS analysis. Because the founding queens of three of the six colonies were used in another study, we used the remaining three queens for analysis. In order to see the possible differences in hydrocarbons between the sexes in a nest, 10 males of the colony were also analyzed.

3. GC-MS analysis

The wasps' hydrocarbons were extracted individually by 1.5 ml *n*-hexane for 10 sec at room temperature. The extracts were concentrated by rotary evaporator, and then the samples were dissolved in 0.2 ml hexane, and the aliquots (0.5%) were analyzed by GC-MS. GC-MS analyses were carried out on a HP 6890N GC, equipped with a nonpolar capillary column DB1-HT (30m×0.25mmI.D.×0.1µm film thickness, J&W Scientific, USA). Helium was the carrier gas, and the column was connected to an Agilent 5973MSD mass selected detector (70eV, Ion Source temperature: 318ºC, Q-poll temperature: 250ºC, Interface: 300). The components were characterized by determining their equivalent chain length and by the analysis of their mass spectra. Quantitation was based on the integration of the total ion chromatograms.

4. Statistical analysis

We followed Butts *et al.* (1995)[3] for the method of hydrocarbon profile analysis. From the results of the GC-MS analysis of the surface hydrocarbons, a percent composition matrix was created for the examined individuals (120 workers, 3 queens, and 10 males). We conducted canonical discriminant analysis to determine whether the cuticular hydrocarbons could be used to discriminate wasps into discrete groups by nestmate, by caste, or by sex. All statistical analyses including the canonical discriminant analysis were done with Systat Ver. 9.01 for Windows (SPSS Inc., 1998).

Results

A total of 35 peaks of hydrocarbons were detected with the GC-MS analysis of the cuticular hydrocarbons of *V. analis* workers, queens, and males, and 34 of the 35 peaks were subsequently identified (Fig. 1, Table 1). The components were n-alkanes, alkens, methyl-alkanes, and dimethyl-alkanes with ECL (equivalent chain length) varying from 21.00 to 37.52. The average proportions (percent to total) of the components are given in Table 1. The largest proportion was occupied by C25 in all wasps, followed by 13MeC27 in workers and males, and by C27 in queens. Canonical discriminant analysis was performed on 120 workers (20 from each of the six colonies) using the proportions of the 35 components as variables in order to group the wasps into colonies. Forward stepwise analysis (limit of variable entry set at F>=4) showed that all but one of the 120 workers were correctly classified into their respective colonies by canonical discriminant functions using 28 components as variables (Table 2, Fig. 2). The five principle components that contributed most to the discrimination were 3MeC25, followed by 13+15MeC31, C25, C23, and 3MeC31. A similar discriminant analysis was performed on a total of 123 wasps after three queens were added to the above workers. The three queens were correctly allocated to their respective colonies as well as the workers, except, again, for one worker (Fig. 2). The components responsible for discrimination were slightly different from those in which only the workers were included: the five principle components were C23, 9C29:1, 3MeC31, C25, and 3MeC25 in order of *F* value in the final discriminant model.

In order to see whether queens and workers could be divided into two groups based on hydrocarbons, canonical discriminant analysis was performed on the 123 wasps, which comprised of three queens and 120 workers. The analysis resulted in two discrete categories of queens and workers (Fig. 3). The five components that most contributed to the discrimination were 12+14MeC26, 13MeC37, 3MeC27, C26, and DiMeC37 (Table 2). These key components did not overlap with the components that contributed to the intercolonial differences of workers or females (workers and queens).

Lastly, we performed another discriminant analysis on 10 males and 20 workers of the same colony to examine the sexual differences in hydrocarbon compositions. They were also clearly discriminated (Fig. 4), and the compounds most responsible for the discrimination were C29, C25:1, 13+15MeC31, and 5MeC25 (Table 2).

Discussion

Previous studies of hydrocarbon profiles in social wasps have identified 13[1] or 19[16] components in *V. crabro*, 14 in *Dolichovespula maculata*[2], 20 in *P. fuscatus*[7], 22 in *P. exclamans*[18], and 25 in *P. metricus*[10] in North America, and 63 in *P. biglumis bimanculatus*[11,12] and 50 in *P. dominulus*[5] in Europe. As compared with North American species, European species had a much larger number of components, and *V. analis* had a relatively larger number of components, 35 (Fig. 1). The number of hydrocarbon components significantly differed between the congeners, *V. analis* and *V. crabro*[3]. This showed that the number of components could vary substantially even within a genus.

V. analis had many components that are not found in *V. crabro*[3,16]. The main hydrocarbon components were similar but slightly different for the two species. The component with the largest proportion was pentacosene (C25:1) in *V. crabro*[3,16], and pentacosane (C25) in *V. analis* (Table 1, 2).

Canonical discriminant analyses showed that the hydrocarbon profiles of *V. analis* queens and workers were different among colonies, as in the case of *P. dominulus*[20], and at the same time, queens formed a group distinct from workers (Fig. 2). The hydrocarbon components responsible for discrimination among colonies were different from those for caste discrimination. In addition, males had different profiles from workers of the same colony (Fig. 4).

Although the number of components of cuticular hydrocarbons and their composition were different, these results were very similar to those reported for *V. crabro*[3]. The results of the GC-MS analyses showed 12 major components and 20 minor components (Table 1, 2). The results showed some intraspecific variation in the quantities of each component of the profiles, even within the same colony or the same cast.

In conclusion, in the case of the common Japanese hornet, *V. analis* has colony-specific cuticular hydrocarbon profiles, but the number of identified compounds is much larger when compared with its congener, *V. crabro*, in Europe. We also found that queens have hydrocarbon profiles different from those of workers, and the hydrocarbon profiles of males differed from those of workers of the same colony. However, this report is a step towards a better understanding of the role of cuticular hydrocarbons in Asian social wasps. Further study is necessary to determine the details of the chemical system of nestmate recognition.

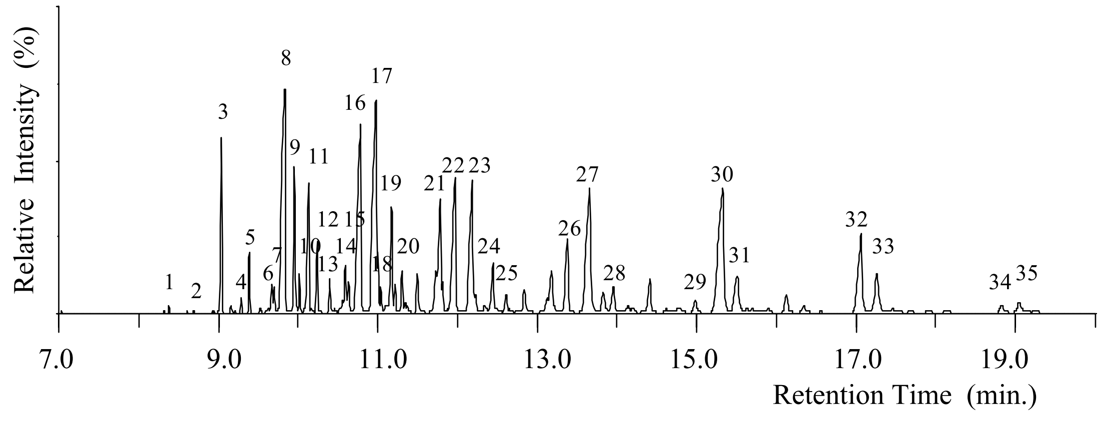

Fig. 1. Total ion chromatograms of cuticular hydrocarbons of a *Vespa analis* worker in Japan

Acknowledgments

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Table 1. Mean percent of composition and standard deviation of cuticular hydrocarbon components in workers, queens and males of *Vespa analis*

peak No.	ECL[1)]	Mean of workers (N=120)	SD[2)] of workers	Mean of queens (N=3)	SD of queens	Mean of males (N=10)	SD of males	component
1	21.00	0.41	0.41	0.21	0.13	0.88	0.41	*n*-C21
2	22.00	0.07	9.00	0.07	0.07	0.15	0.04	*n*-C22
3	23.00	4.51	2.62	5.91	1.65	5.67	0.91	*n*-C23
4	23.70	0.29	0.65	0.27	0.21	0.32	0.04	3MeC23
5	24.00	0.95	0.31	1.97	0.27	1.37	0.15	*n*-C24
6	24.66	0.69	0.52	0.13	0.11	1.62	0.54	C25:1a
7	24.73	1.10	0.98	0.62	0.27	0.59	0.15	C25:1b
8	25.00	12.63	4.49	22.43	3.98	14.50	1.50	*n*-C25
9	25.30	3.26	1.54	2.05	0.80	3.87	0.56	11+13MeC25
10	25.47	0.46	0.21	0.49	0.18	0.34	0.05	5MeC25
11	25.72	2.47	0.87	3.81	1.17	2.83	0.55	3MeC25
12	26.00	1.07	0.47	2.41	0.34	0.97	0.21	*n*-C26
13	26.31	0.65	0.46	0.47	0.17	0.69	0.10	12+13MeC26
14	26.68	0.77	0.71	0.19	0.16	2.06	0.25	C27:1a
15	26.76	1.68	1.24	1.58	0.56	0.63	0.08	C27:1b
16	27.00	8.48	2.96	17.25	2.16	6.34	0.91	*n*-C27
17	27.33	10.62	2.76	7.38	1.86	12.36	1.69	13MeC27
18	27.49	0.42	0.23	0.41	0.06	0.28	0.04	5MeC27
19	27.73	2.52	1.30	4.74	0.50	2.67	0.73	3MeC27
20	28.00	0.72	0.42	1.26	0.14	0.38	0.08	*n*-C28
21	28.73	4.33	2.33	2.33	0.87	3.94	0.38	9C29:1
22	29.00	4.42	1.81	5.43	0.11	2.57	0.19	*n*-C29
23	29.32	4.53	1.47	2.38	0.92	5.44	0.47	13+15MeC29
24	29.73	0.93	0.41	1.30	0.10	0.84	0.31	3MeC29
25	30.00	0.35	0.19	0.16	0.05	0.14	0.02	*n*-C30
26	31.00	1.62	0.69	0.78	0.23	0.92	0.15	*n*-C31
27	31.31	4.58	1.43	1.51	0.55	4.87	0.25	13+15MeC31
28	31.72	0.45	0.21	0.27	0.04	0.40	0.10	3MeC31
29	33.00	0.23	0.14	0.10	0.03	0.17	0.04	*n*-C33
30	33.35	4.94	2.11	1.48	0.33	4.41	0.33	13MeC33
31	33.57	1.47	0.65	0.38	0.18	1.36	0.19	DiMeC33
32	35.32	2.29	0.99	0.85	0.22	2.04	0.23	13MeC35
33	35.55	1.66	0.74	0.46	0.23	1.55	0.18	DiMeC35
34	37.28	0.22	0.13	0.28	0.10	0.32	0.08	13MeC37
35	37.52	0.51	0.23	0.20	0.18	0.62	0.07	DiMeC37

1) Equivalent chain length.
2) Standard deviation.

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Table 2. Rankings of components responsible for discrimination of various categories using forward stepwise canonical discriminant analysis. Ranks are in order of F value in the final discriminant model (F>=4)

Component	Categories of discrimination			
	Workers by colony	Females by colony[1)]	Workers vs. queens	Workers vs. males[2)]
n-C21	27	22		
n-C22				6
n-C23	4	1		5
3MeC23	12	9		
n-C24	7	7		9
C25:1a	16	20		
C25:1b	15	21	14	2
n-C25	3	4		
11+13MeC25	23	24		
5MeC25	22	18		4
3MeC25	1	5		
n-C26	24	23	4	
12+13MeC26	11	14	1	
C27:1a				
C27:1b	25		10	10
n-C27			12	
13MeC27	6			
5MeC27				8
3MeC27	13	13	3	
n-C28	21	25	13	
9C29:1	20	2		
n-C29		26	8	1
13+15MeC29	18	10		
3MeC29	8	6		
n-C30	19	15		
n-C31	14	12	15	
13+15MeC31	2	16		3
3MeC31	5	3	11	
n-C33	28		9	
13MeC33	10	17	6	
DiMeC33	26	27	7	
13MeC35	9	11		
DiMeC35	17	19		11
13MeC37		8	2	
DiMeC37			5	7

1) Females contained both workers and queens.
2) Workers and males of a single colony.

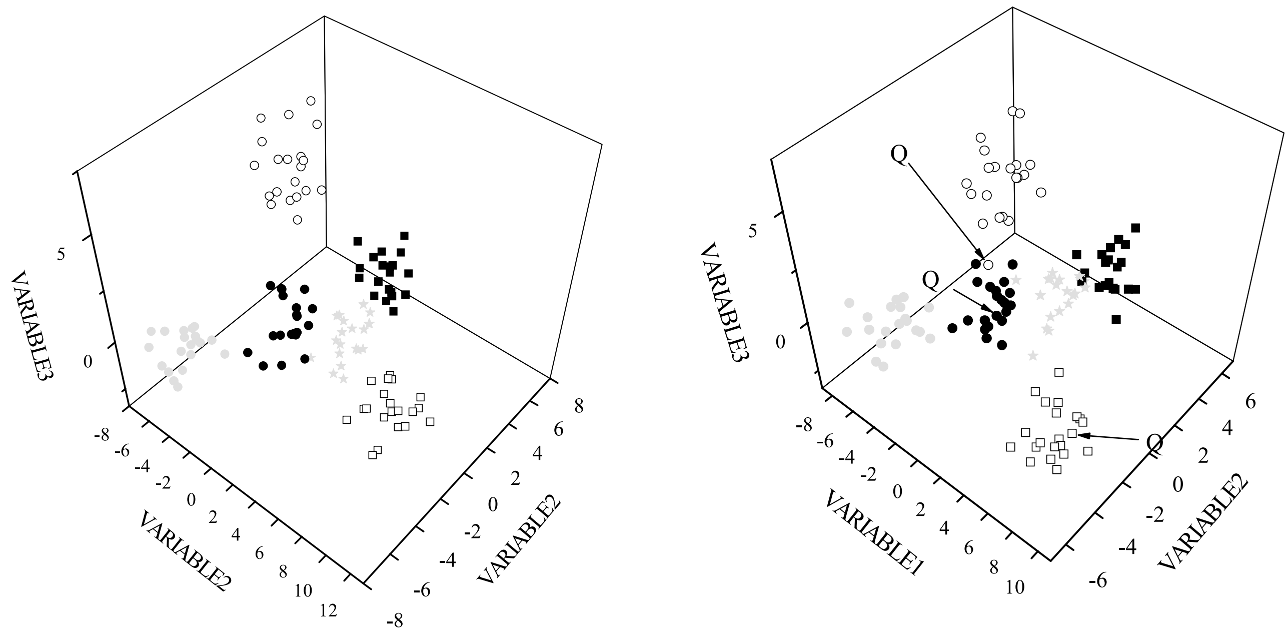

Fig. 2. Three-dimensional plots of the first three discriminant variables from a canonical discriminant analysis of cuticular hydrocarbons of *Vespa analis*

Left: 120 workers from six colonies.

Right: 120 workers from six colonies plus three queens in three of the six colonies.

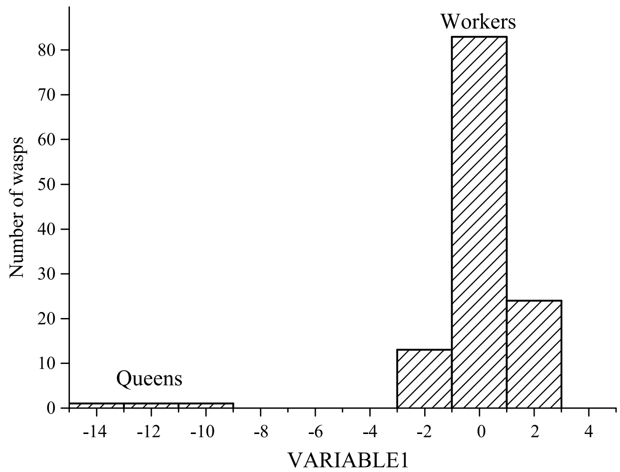

Fig. 3. Frequencies of the discriminant variable from a discriminant analysis of the hydrocarbons from 120 workers of six colonies and queens of three of the six colonies

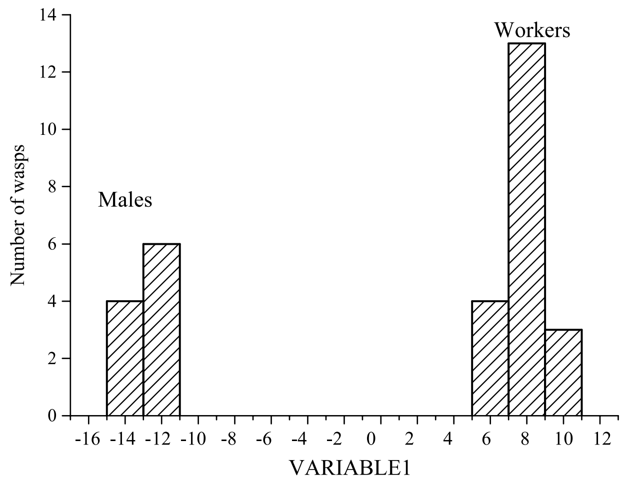

Fig. 4. Frequencies of the discriminant variable from a discriminant analysis of the hydrocarbons from 20 workers and 10 males of a single colony of *Vespa analis*

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