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CASTE AND POPULATION SPECIFICITY OF TERMITE CUTICULE HYDROCARBONS

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The composition of hydrocarbons from the termites Reticulitermes lucifugus *Rossi and* Kalotermes flavicollis *Fabr. belonging to different castes and populations was determined using GC and mass spectrometry. The role of cuticular hydrocarbons in olfactory recognition of termite caste and population status was demonstrated.*

Key words: *Reticulitermes lucifugus* Rossi, *Kalotermes flavicollis* Fabr., cuticular hydrocarbons, caste and population specificity.

Insect cuticular hydrocarbons play an exceptionally important role in their lives. They are a component part of the insect exoskeleton and determine many of its properties [1]. Much attention is directed toward the role of cuticular hydrocarbons in insect behavior. Cuticular hydrocarbons are especially significant in social insects [2-4], which distinctly differentiate the caste and gender status of individuals [5]. Since the majority of social insects, and termites in particular, live in soil or wood, chemical communication among them is of utmost importance [6]. The nests and tubules of termites are enclosed and their volume is relatively small. Under such conditions, chemical signals should have the ability to transfer large quantities of information and should be relatively involatile in order to decrease the noise levels of information transfer so that the chemical-communication system operates effectively. Therefore, termite cuticular hydrocarbons should play an important role in olfactory recognition of the caste, population, and species status of individuals [3, 7-9].

The composition of cuticular hydrocarbons of *Kalotermes flavicollis* Fabr. and *Reticulitermes lucifugus* Rossi was determined by GC and GC—MS (Tables 1 and 2). The structures of all compounds were established using analytical GC and mass spectrometry. The cuticular hydrocarbons of the studied termite species belong to five classes: normal alkanes, mono- and dimethylalkanes, and mono- and dienes. The hydrocarbon composition of these termite species has much in common and is similar to that of cuticular hydrocarbons of other species [7].

We found that the cuticular hydrocarbons of both termite species contained the same set of hydrocarbons for all castes. However, they differed greatly in the quantitative ratios of both pure components and hydrocarbon classes (Table 3).

Analysis of the composition of *R. lucifugus* cuticular hydrocarbons from various populations showed that termites of all populations also contain qualitatively identical components whereas the quantitative ratios of them, like for termites of different castes, differ (Table 4). The differences in the ratios of different clases are greatest for termites of the Ai-Dere and Magri populations.

Our investigation confirms the existence of caste and population specificity for cuticular hydrocarbons of *R. lucifugus*. The observed differences in the compositions of cuticular hydrocarbons may be the basis for olfactory recognition of the caste and population status of individuals.

In order to prove that the specificity of cuticular hydrocarbons is the basis of olfactory recognition of the caste and population status of individuals, we used the recognition reaction of termites from various populations that is manifested as touching of antennae of "foreign" termites. The situation was modeled using castings of termites treated with hydrocarbons from various termite populations.

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TABLE 1. Cuticular Hydrocarbons of *K. flavicollis*

Hydrocarbon	Equivalent chain length	Diagnostic MS ions (70 eV, *m/z*), I_{rel} %
n-C_{20}	20.00	282(M^+, 6)
n-C_{21}	21.00	296(M^+, 5)
n-C_{22}	22.00	310(M^+, 3)
n-C_{23}	23.00	324(M^+, 5)
5-MeC_{23}	23.50	168/169; 196/197; 323; 338(M^+, 3)
3-MeC_{23}	23.70	294/295; 323/324; 338(M^+, 3)
n-C_{24}	24.00	338(M^+, 4)
2-MeC_{24}	24.70	308/309 (75); 336/337 (16); 352 (M^+, 5)
n-C_{25}	25.00	352(M^+, 6)
5-MeC_{25}	25.40	85(100); 280/281(11); 308/309(48); 351(4); 366(M^+, 7)
3-MeC_{25}	25.50	57(100); 308/309(10); 336/337(54); 351(4); 366(M^+, 4)
n-C_{25}:2	25.60	124(21); 138(15); 277/278(4); 291/292(6); 348(M^+, 10)
2-MeC_{25}	25.70	322/323(61); 350/351(13); 366(M^+, 4)
n-C_{26}	26.00	366(M^+, 5)
n-C_{27}	27.00	380(M^+, 2)
3-MeC_{27}	27.5	336/337(13); 364/365(62); 379(4); 394(M^+, 5)
n-C_{29}	29.0	408 (M^+, 4)
2-MeC_{29}	29.7	378/379(54); 406/407(3); 422(M^+, 2)
diMeC_{29}	31.5	168/169(39); 322/323(16); 336/337(19); 364/365(11); 464(M^+, 2)
diMeC_{33}	33.4	168/169(46); 350/351(16); 364/345(21); 392/393(9); 492(M^+, 3)
11,15-diMeC_{35}	35.5	168/169(43); 378/379(15); 392/393(17); 420/421(8); 520(M^+, 2)
2-MeC_{39}	39.7	518/519(61); 546/547(4); 562(M^+, 2)

TABLE 2. Cuticular Hydrocarbons of *R. lucifugus*

Hydrocarbon	Equivalent chain length	Diagnostic MS ions (EI, 70 eV), *m/z* (I_{rel} %)
n-C_{20}	20.00	282(M^+, 6)
n-C_{21}	21.00	296(M^+, 7)
3-MeC_{21}	21.65	57(100); 253/254(7); 281/282(45); 310(M^+, 6)
n-C_{22}	22.00	310(M^+, 3)
n-C_{23}:1	22.30	223(11); 237(9); 322(M^+, 10)
n-C_{23}:2	22.50	152(16); 166(14); 221(14); 235/236(10); 320(M^+, 12)
n-C_{23}	23.00	324(M^+, 15)
5-MeC_{23}	23.40	85(100); 253/254(11); 281/282 (42);338(M^+, 3)
2-MeC_{23}	23.70	294/295(60); 323/324(8); 338(M^+, 3)
n-C_{24}	24.00	338(M^+, 4)
3-MeC_{24}	24.65	57(100); 295/296(7); 323/324(45); 352(M^+, 6)
2-MeC_{24}	24.70	308/309 (79); 336/337 (16); 352 (M^+, 4)
n-C_{25}:1	24.30	251(12); 265(10); 350(M^+, 12)
n-C_{25}	25.00	352(M^+, 6)
5-MeC_{25}	25.40	85(100); 280/281(11); 308/309(53); 351(4); 366(M^+, 4)
3-MeC_{25}	25.50	56/57(100); 308/309(10); 336/337(65); 351(4); 366(M^+, 4)
n-C_{25}:2	25.60	124(23); 138(16); 277/278(4); 291/292(7); 348(M^+, 12)
n-C_{26}	26.00	366(M^+, 5)
3-MeC_{26}	26.50	322/323; 350/351(68); 365(3); 380(M^+, 3)
2-MeC_{26}	26.70	336/337(55); 365(5); 380(M^+, 5)
n-C_{27}	27.00	380(M^+, 2)

TABLE 3. Cuticular Hydrocarbons of *K. flavicollis* and *R. lucifugus* and Their Distribution by Caste (%, M ± m, P = 0.95)

K. flavicollis hydrocarbon	Distribution by caste			
	Pseudoergates	Nymphs	Soldiers	Winged imagoes
$n\text{-C}_{20}$	<0.5	<0.5	<0.5	<0.5
$n\text{-C}_{21}$	1.4±0.1	1.2±0.1	0.6±0.1	1.1±0.1
$n\text{-C}_{22}$	0.6±0.1	<0.5%	<0.5	<0.5%
$n\text{-C}_{23}$	1.2±0.1	1.2±0.1	1.4±0.1	1.6±0.1
5-MeC_{23}	5.0±0.2	3.3±0.1	2.2±0.2	3.5±0.2
3-MeC_{23}	1.5±0.2	<0.5	<0.5	<0.5
$n\text{-C}_{24}$	1.2±0.2	1.8±0.1	0.7±0.1	1.8±0.1
2-MeC_{24}	<0.5	<0.5	<0.5	<0.5
$n\text{-C}_{25}$	20.3±0.5	15.1±0.4	8.9±0.3	15.0±0.3
5-MeC_{25}	0.6±0.1	1.6±0.1	1.3±0.1	1.6±0.1
3-MeC_{25}	10.2±0.3	8.4±0.2	11.4±0.2	8.2±0.3
$n\text{-C}_{25}{:}2$	7.2±0.1	10.1±0.2	10.7±0.2	10.2±0.3
2-MeC_{25}	0.8±0.2	<0.5	<0.5	<0.5
$n\text{-C}_{26}$	5.5±0.2	8.4±0.2	10.8±0.3	8.2±0.2
$n\text{-C}_{27}$	3.1±0.1	3.0±0.1	3.1±0.1	3.1±0.1
MeC_{27}	2.2±0.1	2.2±0.1	2.2±0.1	2.2±0.1
$n\text{-C}_{29}$	3.1±0.2	3.2±0.2	3.1±0.1	3.2±0.1
MeC_{29}	<0.5	<0.5	<0.5	<0.5
diMeC_{31}	16.4±0.3	18.5±0.3	21.5±0.4	17.7±0.5
diMeC_{33}	14.3±0.2	17.4±0.5	17.2±0.3	17.3±0.4
diMeC_{35}	1.6±0.1	<0.5	<0.5	<0.5
2-MeC_{39}	1.7±0.1	<0.5	0.7±0.1	<0.5
R. lucifugus hydrocarbon	Workers	Nymphs	Soldiers	Winged imagoes
$n\text{-C}_{20}$	<0.5	<0.5	<0.5	<0.5
$n\text{-C}_{21}$	<0.5	<0.5	<0.5	<0.5
3-MeC_{21}	1.7±0.1	<0.5	<0.5	<0.5
$n\text{-C}_{22}$	<0.5	<0.5	<0.5	<0.5
$n\text{-C}_{23}{:}1$	<0.5	<0.5	<0.5	<0.5
$n\text{-C}_{23}{:}2$	<0.5	<0.5	<0.5	<0.5
$n\text{-C}_{23}$	34.0±0.5	33.8±0.4	23.0±0.2	27.0±0.3
5-MeC_{23}	3.0±0.1	5.2±0.2	3.4±0.1	4.4±0.2
2-MeC_{23}	0.7±0.1	<0.5	<0.5	<0.5
$n\text{-C}_{24}$	4.2±0.2	6.8±0.1	6.1±0.1	3.7±0.1
3-MeC_{24}	1.2±0.1	3.8±0.2	1.4±0.1	1.9±0.1
2-MeC_{24}	12.6±0.2	10.4±0.2	16.5±0.1	25.2±0.2
$n\text{-C}_{25}{:}1$	8.3±0.2	6.5±0.1	8.4±0.2	4.8±0.2
$n\text{-C}_{25}$	6.4±0.3	8.3±0.1	6.3±0.1	6.2±0.1
5-MeC_{25}	9.2±0.2	6.1±0.2	11.3±0.1	7.1±0.1
$n\text{-C}_{25}{:}2$	3.1±0.1	2.6±0.1	10.2±0.2	3.4±0.1
3-MeC_{25}	1.2±0.2	2.3±0.1	1.7±0.1	2.6±0.2
$n\text{-C}_{26}$	3.4±0.2	4.6±0.1	2.1±0.1	3.4±0.1
3-MeC_{26}	1.4±0.1	0.7±0.1	0.7±0.1	0.8±0.1
2-MeC_{26}	1.6±0.1	1.4±0.1	1.1±0.1	1.8±0.1
$n\text{-C}_{27}$	3.3±0.1	2.4±0.1	2.1±0.1	2.4±0.1

TABLE 4. Cuticular Hydrocarbons of *R. lucifugus* of Various Populations (%, M ± m, P = 0.95)

Hydrocarbon	Hydrocarbon content in termite populations					
	Alekseevka	Ai-Dere	Barda	Gasmalyan	Megri	Odessa
n-C$_{20}$	<0.5	1.3±0.1	0.7±0.1	0.8±0.1	<0.5	0.8±0.1
n-C$_{21}$	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
3-MeC$_{21}$	1.8±0.1	<0.5	0.5±0.1	<0.5	<0.5	<0.5
n-C$_{22}$	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
n-C$_{23}$:1	<0.5	<0.5	<0.5	<0.5	<0.5	-
n-C$_{23}$:2	<0.5	<0.5	<0.5	<0.5	4.2±0.1	2.4±0.2
n-C$_{23}$	33.0±0.2	<0.5	1.4±0.1	<0.5	54.0±0.4	20.0±0.2
5-MeC$_{23}$	3.0±0.1	<0.5	<0.5	<0.5	<0.5	<0.5
2-MeC$_{23}$	0.7±0.1	3.7±0.1	1.8±0.1	1.7±0.1	<0.5	2.2±0.1
n-C$_{24}$	4.2±0.1	<0.5	<0.5	<0.5	<0.5	2.6±0.1
3-MeC$_{24}$	1.2±0.1	<0.5	<0.5	<0.5	<0.5	-
2-MeC$_{24}$	12.6±0.1	20.0±0.1	17.0±0.2	17.8±0.1	5.4±0.1	10.3±0.1
n-C$_{25}$:1	8.3±0.1	5.5±0.1	4.8±0.1	5.8±0.1	0.8±0.1	6.3±0.1
n-C$_{25}$	6.6±0.1	4.4±0.1	0.6±0.1	2.5±0.1	2.6±0.1	1.2±0.1
5-MeC$_{25}$	9.6±0.1	<0.1	50.2±0.4	45.0±0.4	9.5±0.3	33.0±0.5
n-C$_{25}$:2	3.1±0.1	43.2±0.5	5.1±0.1	4.9±0.1	10.2±0.1	4.5±0.1
3-MeC$_{25}$	1.2±0.1	9.2±0.1	<0.5	<0.5	5.2±0.1	<0.5
n-C$_{26}$	3.6±0.1	6.5±0.1	7.5±0.2	11.6±0.3	<0.5	10.3±0.2
3-MeC$_{26}$	1.7±0.1	<0.5	0.7±0.1	<0.5	<0.5	<0.5
2-MeC$_{26}$	1.8±0.1	<0.5	<0.5	<0.5	<0.5	<0.5
n-C$_{27}$	3.8±0.1	<0.5	4.1±0.1	2.6±0.1	<0.5	<0.5

TABLE 5. Effect of Cuticular Hydrocarbons on Recognition Reaction of *R. lucifugus* of Various Populations (Number of "Casting" Antennae Touches per Minute, %, M ± m, P = 0.95)

Reaction of termite population	Termite castings treated with termite-population hydrocarbons					
	Alekseevka	Ai-Dere	Barda	Gasmalyan	Megri	Odessa
Alekseevka	4.7±1.5	7.7±2.3	7.1±1.8	7.1±1.8	7.6±1.4	6.1±1.8
Ai-Dere	7.8±1.4	4.2±0.9	7.4±0.8	7.2±0.7	5.6±1.7	7.4±0.8
Barda	7.4±0.7	6.8±1.2	4.4±2.4	4.3±1.4	5.2±0.9	4.5±1.4
Gasmalyan	7.2±0.6	5.8±1.2	4.5±1.4	4.8±1.6	5.1±1.4	4.4±1.2
Megri	7.1±0.8	7.3±1.3	7.4±0.9	7.3±0.9	4.9±1.5	4.2±1.2
Odessa	4.9±1.4	7.8±1.4	5.6±1.3	5.2±2.3	5.3±1.8	5.1±1.9

The biological tests showed that, in spite of the identical set of cuticular hydrocarbons, their ratios had a significant effect on recognition of the population status of *R. lucifugus* (Table 5). Thus, termites of the Alekseevka, Ai-Dere, and Megri populations touched more frequently castings with cuticular hydrocarbons of termites from other populations than those with hydrocarbons from termites of their own population (the difference was statistically significant with P = 0.95). Termites with a similar composition of cuticular hydrocarbons did not differentiate the castings from each other in this test, for example, termites of the Barda and Gasmalyan populations.

Thus, it was demonstrated that termites use cuticular hydrocarbons for recognition of population status. The difference in compositions of cuticular hydrocarbons of termites in different castes and species (Tables 1-3) [7] indicates that cuticular hydrocarbons are used in this instance for recognition of individuals.

We propose that the composition of cuticular hydrocarbons is the basis for chemical recognition of termites of a single species, population, caste, etc. Confirmation of this can be found in the literature [4, 8, 10, 11]. The mechanism causing the difference in the compositions of cuticular hydrocarbons is probably the biosynthetic system. Another mechanism causing the specificity of termite cuticular hydrocarbons is their habitat and food because termites can rather quickly adsorb hydrocarbons [12].

EXPERIMENTAL

We used *R. lucifugus* Rossi (Isoptera, Rhinotermitidae) and *K. flavicollis* Fabr. (Isoptera, Kalotermitidae) in the experiments. *R. lucifugus* was collected near Barda village (Azerbaidzhan), Barda population; Gasmalyan village (Azerbaidzhan), Gasmalyan population; Alekseevka village (Azerbaidzhan), Alekseevka population; and near Megri village (Armenia), Megri population; Ai-Dere canyon (West Kopetdag, Turkmenistan), Ai-Dere population; and in Odessa, Odessa population. *K. flavicollis* were collected on Myussersk reservation in Abkhaziya.

Cuticular hydrocarbons were extracted three times by immersing 30 insects in hexane (10 mL). The portions were combined, concentrated, and used in further investigations.

Cuticular hydrocarbons were separated from other lipids by chromatography in hexane over a silica-gel column (L 100-200 mesh, 2.0 g). The hydrocarbon fraction was analyzed by GC (Chrom-5) using a flame-ionization detector, quartz capillary column (0.1 mm × 25 m) with SE-30 stationary phase, and a 1:100 ratio. Cuticular hydrocarbons were determined quantitatively using an on-column injection method with vaporizer and detector temperatures of 300°C, programmed column temperature from 150 to 300°C at 6°C/min with a 4-minute hold at 150°C and a 10-minute hold at 300°C. The retention times were compared with *n*-alkane standards. Mass spectra were recorded in a Finnigan 4021 GC—MS at 70 eV using a quartz capillary column (0.1 mm × 25 m) and SE-30 stationary phase. The structures of the hydrocarbons were determined using GC, GC—MS, and literature data [7, 13].

Hydrocarbons were designated using abbreviations proposed in the literature [8] where Me indicates methyl; C_{xx}, the number of C atoms in the molecule excluding methyl; and C_{xx}:Y, the number of double bonds in the molecule. Thus, *n*-pentacosane is written as *n*-C_{25}; methylpentacosane, Me-C_{25}; pentacosadiene, C_{25}:2.

The role of cuticular hydrocarbons in termite chemical communication was investigated using castings that were prepared as before [7]. Termite castings of various populations were treated with acetone containing one "termite-equivalent" of cuticular hydrocarbons. The castings were dried in a stream of nitrogen for 1 h before the biological tests. Then, one casting was placed in a terrarium (100 × 100 mm) in as natural a pose as possible. Workers (29) and one soldier were introduced. Their behavior was observed. The number of touches and the interactions of the termites with the casting were recorded.

All experiments were repeated at least ten times. The results were calculated using the Student criterion [14].

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