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Honeybees discriminate cuticular waxes based on esters and polar components

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Abstract – Quantitative chemical analyses of cuticular waxes of the honeybee *Apis mellifera* with gas chromatography and mass spectrometry showed significant differences in the chemical composition of cuticular waxes from drones and workers performing different tasks. We used the proboscis extension reflex to test the ability of bees to discriminate between these cuticular waxes. Differentially conditioned bees significantly discriminated between cuticular waxes of drones, food storers, foragers and queen attenders. We found that the esters and polar components in the cuticular waxes provide the discriminative cues for the insects.

***Apis mellifera* / cuticular wax / chemical component / proboscis extension reflex / differential conditioning / discrimination of wax**

1. INTRODUCTION

Aliphatic hydrocarbons in the cuticular waxes of bees are widely assumed to function as recognition cues (for nestmate and for kin) used in intracolonial interactions between individuals (McDaniel et al., 1984; Francis et al., 1985; Page et al., 1991; Arnold et al., 1996). Quantitatively, the aliphatic hydrocarbons constitute the largest fraction of cuticular wax in bees and cover a highly variable spectrum of chain lengths and saturation (Breed et al., 1992). The components of this spectrum vary amongst groups of bees in a colony that can be distinguished on other grounds such as their kin relationship and the behavioural tasks they perform (Francis et al., 1989;

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Page et al., 1991; Fröhlich et al., 2000b). It has therefore been assumed that aliphatic hydrocarbons may label individuals in colonies according to the tasks they undertake and that the compounds provide a chemical basis for kin recognition (Getz et al., 1991; Breed et al., 1992). Accordingly, research on the ability of insects to discriminate between different waxes has focussed on hydrocarbons (Howard, 1993; Smith and Breed, 1995) although only very few studies have shown that insects respond directly to hydrocarbons (Breed, 1998; Lahav et al., 1999). Nevertheless it is assumed that insects can distinguish sexes and castes on the basis of the differences in wax composition (Fröhlich et al., 2000b). The chemical profiles of the bee cuticular waxes, though, are rather more dynamic than static and the environment of the bees has an important influence on this profile (Howard, 1993). It is, therefore, conceivable that the cuticle of individuals that undertake different tasks, like food storer bees, foragers or queen attendants, may exhibit different composition of cuticular wax and so be recognized as such by nestmates.

Bees can be trained to extend the proboscis when provided with different chemical stimuli and rewarded with sugar (Menzel et al., 1993). In a previous study we have used this proboscis extension reflex (PER) to show that bees learn to distinguish between honey comb waxes of different ages, defined by their chemical composition (Fröhlich et al., 2000b). The bees use the polar components of wax in this discrimination, not the aliphatic hydrocarbons (Fröhlich et al., 2000a). We have now exploited the PER behaviour to test the abilities of bees to discriminate between different cuticular waxes.

2. MATERIALS AND METHODS

Drones and worker bees of *Apis mellifera* engaged in different tasks (foragers, food storer bees, queen attendants) were taken from one single colony and killed by immersion in liquid N_2. Waxes were extracted from a sample of 20 bees by immersing killed bees in chloroform ($CHCl_3$) for approx. 30 s at room temperature. For drones and food storer workers, whole wax extracts were processed using a solid phase extraction method described previously (Nass et al., 1998). Five fractions were obtained: fraction A (eluted with hexane); fraction B (eluted after fraction A with diethylether); fraction C (eluted after fraction A with isopropylchloride); fraction D (eluted after fraction C with diethylether); fraction E (eluted after fraction D with a mixture of $CHCl_3$, methanol and water, 13:5:1 respectively). Fraction B therefore contained the same substances as fraction C and D (B = C+D).

Capillary gas chromatography (5890 series II, Hewlett Packard, Avondale, Pa.) with on-column injection onto a 30 m DB-1 open tubular fused silica column (i.d. 0.32 mm, film 0.1 μm; J&W Scientific, Folsom, Ca.) and flame ionisation or mass selective detection (70 eV, m/z 50–650, Hewlett-Packard 5971) was carried out using the following temperature programme: injection at 50 °C, 2 min at 50 °C, 40 °C/min up to 200 °C, 2 min at 200 °C, 3 °C/min up to 320 °C (FID) or 300 °C (MS), 30 min at 320 °C (300 °C). Carrier gas pressures were adjusted as follows: 30 min at 50 kPa, 10 kPa/min up to 150 kPa for flame ionisation detection (hydrogen) and 30 min at 10 kPa, 10 kPa/min up to 100 kPa for mass selective detection (helium). For the quantification of wax components internal standards were added to the raw extracts. Tetracosane, methyl triacontanoate and tetracosanoic acid were chosen as standards because they co-eluted with each of the wax fractions analysed by gas chromatography.

The cuticular waxes were extracted from bees that came from the same hive as those that were used in the PER tests. Previous experiments have shown that bees learned waxes of the own hive better than waxes of a strange hive. To condition bees for the PER, wax solutions were applied to glass

rods (100–200 μg per rod) by dropping the appropriate wax solution onto the rods. The rods were then turned until the solvent had evaporated. The rods were then stored at –18 °C until use up to two weeks later.

Differential conditioning of the PER followed the method described previously for testing the ability of bees to discriminate between different comb waxes (Fröhlich et al., 2000a). The behavioural tests employed differential training of the PER to whole wax extracts of drones and workers in different task groups. For cuticular waxes of drones and food storer workers four different wax fractions (A, B, C and D) were used in addition to the whole extracts. One type of wax was paired with reward (CS+) and another with non-reward (CS–). Waxes from the same solution on two different rods were used as a non-learning control. The conditioning was performed according to the following learning and testing scheme (Tab. I).

Two repetitions were performed for each experiment with 20 bees, respectively. Three control runs were made. Bees that reacted spontaneously with a proboscis extension in the first trial of the learning phase were excluded from the experiment. Errors per bee in the testing phase were calculated. Frequency histograms of the numbers of individuals that made from 0 to 6 errors were constructed for each group of 35–40 test bees and the medians of errors were calculated. Chi^2-tests were conducted to compare the error histograms of the tests with the control experiments. To minimise the risk of Type I error the following significance levels of $p \leq 0.0001$ significant and $p > 0.0001$ not significant were chosen.

Table I. Learning and testing scheme used in the conditioning of bees (CS+ = conditioning stimulus with reward, CS– = different conditioning stimulus with no reward, US = unconditioned stimulus).

	Trial					
Learning	1	2	3	4	5	6
CS+ and US:	+			+		+
CS– and US:		–	–		–	
Testing	7	8	9	10	11	12
CS+:	+			+		+
CS–:		–	–		–	

3. RESULTS

Quantitative chemical analysis has shown that cuticular waxes of worker bees of different tasks were dominated by alkyl esters (34–39%) followed by alkenes (15–18%) and alkanes (14–17%) (Tab. II). Discriminant function analysis of cuticular wax compositional data (Tab. II) yielded five variables that allow to distinguish significantly between cuticular waxes of workers performing different tasks: unsaturated alkyl esters, alkadienes, alcohols, alkyl esters and acids. By analyzing cuticular waxes of drones and food storer bees with solid phase extraction the following fractions can be obtained. Fraction A (32–35% of the total cuticular wax mass) consists of unsaturated and saturated, unbranched and branched aliphatic hydrocarbons. Fraction C contains alkyl esters and unsaturated alkyl esters and constitutes 21–30% of the total wax mass. Fraction D makes up only 2–4% of the total wax mass and contains hydroxy alkyl esters, primary alcohols and acids (Tab. III). Fraction E (31–43% of the total wax mass) is comprised of the most polar components, which are not yet amenable to gas chromatography (Fröhlich et al., 2000b). By analyzing cuticular waxes isolated from drones and food storer bees with discriminant function analyses, these waxes could be significantly discriminated by the authors using the relative amounts of substance classes of the total mass of fraction A, C and D.

We trained bees using the PER conditioning according to the learning and testing

scheme (Tab. I) with different cuticular waxes and fraction of waxes in different combinations. Each wax and each fraction was used and tested as a positive and negative conditioning stimulus. With significant differences between the experiment and the control it was evident that the bees could discriminate between the waxes.

Table II. Relative chemical composition of cuticular waxes from workers of different tasks. The relative amounts of masses in % of the whole wax extracts are given (means and 95% confidence intervals; limit of detection at 0.01%, decimals were set accordingly).

Substance classes	Food storer bees	Foragers	Queen attenders
		(n = 6)	
Alkanes	17 ± 7.3	15 ± 3.8	14 ± 3.2
Alkenes	16 ± 2.7	15 ± 3.5	18 ± 6.4
Alkadienes	3.0 ± 0.87	2.4 ± 0.57	3.9 ± 0.94
Branched alkanes	0.97 ± 0.215	0.94 ± 0.376	1.3 ± 0.69
Alkyl esters	34 ± 8.7	39 ± 6.9	35 ± 9.7
Unsaturated alkyl esters	12 ± 1.9	17 ± 3.2	12 ± 2.9
Hydroxyalkyl esters	1.8 ± 1.07	1.4 ± 1.64	2.8 ± 2.47
Acids	6.2 ± 1.26	4.3 ± 1.04	6.9 ± 2.14
Unsaturated acids	0.28 ± 0.244	0.04 ± 0.094	0.35 ± 0.261
Alcohols	1.6 ± 0.52	2.3 ± 0.84	1.8 ± 1.16
Unidentified	6.1 ± 4.80	3.6 ± 0.95	4.9 ± 1.50

Table III. Relative chemical composition of cuticular waxes from drones and food storer bees. The relative amounts of masses in % of the total mass of fraction A, C and D are given (means and 95% confidence intervals; limit of detection at 0.01%, decimals were set accordingly). The value "unidentified" is the sum of unidentified substances over all fractions.

Substance classes	Drones	Food storer bees
	(n = 6)	
Fraction A		
Alkanes	21 ± 1.8	19 ± 5.0
Alkenes	21 ± 1.9	23 ± 5.7
Alkadienes	2.5 ± 0.33	7.6 ± 2.22
Branched alkanes	11 ± 1.6	1.4 ± 0.26
Fraction B		
Fraction C		
Alkyl esters	32 ± 4.2	31 ± 6.1
Unsaturated alkyl esters	4.7 ± 0.80	12 ± 4.8
Fraction D		
Hydroxyalkyl esters	2.4 ± 1.22	2.3 ± 1.22
Acids	0.02 ± 0.061	0.17 ± 0.309
Alcohols	1.2 ± 0.57	1.3 ± 0.49
Unidentified	3.8 ± 1.28	2.4 ± 1.1

In our experiments using the PER conditioning the trained bees discriminated significantly between the whole wax extracts of drones against food storers (Tab. IV, Fig. 1), foragers against food storers, foragers against queen attenders, queen

Table IV. Summary of the results of learning experiments with whole extracts and four fractions of cuticular waxes from bees of different sexes using different combinations of positive (CS+) and negative (CS–) conditioned waxes (ns = not significant, all other pairs give significantly different results; upper value = number of tested bees (N); lower value = median of errors (Z_e) for each combination of waxes; control data: N = 125, Z_e = 2.96).

		Drones	
		CS+	CS–
Whole extracts			
Food storers	CS+		38
			2.64
	CS–	34	
		2.17	
Fraction A			
Food storers	CS+		40
			2.95
			ns
	CS–	36	
		2.79	
		ns	
Fraction B			
Food storers	CS+		37
			2.42
	CS–	34	
		2.00	
Fraction C			
Food storers	CS+		40
			2.05
	CS–	38	
		2.50	
Fraction D			
Food storers	CS+		39
			1.78
	CS–	37	
		1.45	

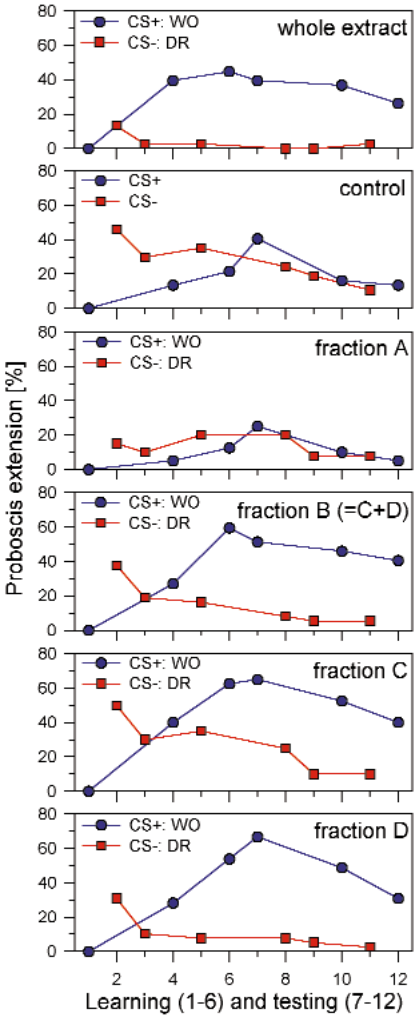

Figure 1. Discrimination curves (n = 20 + 20 bees for each plot) resulting from differential conditioning of the proboscis extension reflex of *Apis mellifera*. Cuticular waxes of workers (WO) and drones (DR) were tested as positive (CS+) and negative (CS–) conditioned waxes using whole extracts and fractions A to D. The distances between the upper and lower curves indicate the ability of the trained bees to discriminate between the tested waxes. Bees do discriminate between waxes using whole extracts and fraction B, C and D, and do not discriminate between waxes using fraction A and in the control experiment (same waxes on two different glass rods). The discrimination patterns were identical using DR as CS+ and WO as CS–.

Table V. Summary of the results of learning experiments with whole extracts of cuticular waxes from bees performing different tasks using different combinations of positive (CS+) and negative (CS–) conditioned waxes (all pairs give significantly different results; upper value = number of tested bees (N); lower value = median of errors (Z_e) for each combination of waxes; control data: N = 50, Z_e = 2.99).

Whole extracts		Foragers		Queen attenders	
		CS+	CS–	CS+	CS–
Food storers	CS+		74 2.70		72 2.58
	CS–	75 2.51		76 1.04	
Queen attenders	CS+		38 1.39		
	CS–	38 1.93			

attenders against food storers and vice versa (Tab. V). The best discrimination could be found by using the queen attenders as a positive conditioning stimulus (Z_e = 1.04, 1.39).

However, the discrimination of the waxes was influenced by the fractions present: the bees were unable to discriminate waxes from drones and workers using fraction A (the aliphatic hydrocarbons) whereas fraction B (B = C + D) gave the same result as the whole extract. Subdivision of fraction B into C and D showed, that the bees could use both fractions C and D to discriminate between waxes (Tab. IV, Fig. 1). The best discrimination occurred by conditioning the bees with fraction D comprising hydroxy alkyl esters, acids and primary alcohols. This discrimination based on the wax fraction D was even better than the discrimination based on the whole wax extracts.

4. DISCUSSION

In a previous study we have shown that the chemical composition of cuticular waxes from bees belonging to different castes and sexes can be differentiated by discriminant analysis (Fröhlich et al., 2000b). Here we show that cuticular waxes of workers performing different tasks also can be discriminated by their chemical compositional data with discriminant function analysis.

For the first time, the ability of honeybees to discriminate cuticular waxes and fractions of waxes with a natural distribution of the components have been tested using the proboscis extension reflex paradigm. Each wax and each fraction was used and tested as a positive and as a negative conditioning stimulus, and the bees learned to react equally well to each wax and each cuticular wax fraction. The assymetric conditioning of the bees by using the cuticular wax of queen attenders as positive conditioning stimulus could be explained by the presence of queen mandibular pheromone in the whole extracts of these waxes. Therefore, the whole extracts of cuticular waxes belonging to queen attenders should be more attractive to the trained bees as the whole extracts of the other workers.

As aliphatic compound profiles show considerable variability (Breed et al., 1995), research on the ability of insects to discriminate waxes has focussed on these compounds, especially on aliphatic hydrocarbons

(Howard, 1993; Smith and Breed, 1995). But honeybees also could be able to use polar substances as discriminating cues, because visualizations of odour representations in the honeybee brain showed, that geraniol, isoamylacetate and hexanol evoke odour-specific glomerular activity patterns in the antennal lobe (Joerges et al., 1997). In our experiments with different fractions of the cuticular waxes we find that bees use the esters and more polar components (saturated and unsaturated alkyl esters, hydroxy alkyl esters, acids and primary alcohols; fractions C and D) of the waxes and not the aliphatic hydrocarbons for discriminating between cuticular waxes of different bees. These results are based on more substance classes than in our previous experiments with comb waxes where the bees used only fraction D (hydroxy alkyl esters, acids and primary alcohols) as a discriminatory cue (Fröhlich et al., 2000a).

Honeybees were able to discriminate different blends of tricosane and pentacosane (Getz and Smith, 1987), but the obvious differences in the alkadienes and alkenes of the aliphatic hydrocarbon fraction found with chemical analyses (Fröhlich et al., 2000b) seemed not to be relevant in enabling the bees to discriminate between the waxes. The mixture of aliphatic hydrocarbons in fraction A may be too complex for the bees to detect the differences. This assumption is based on a neurobiological study (Joerges et al., 1997), where odour mixtures with more than two components led to inhibitory effects in the spatio-temporal excitation patterns in the antennal lobe of the honeybee. More discriminating cues could be present in fraction E, which could contain more acids, alcohols, hydroxyacids, diols and even mono- and diacylglycerols (Davidson and Hepburn, 1986).

The present work provides new insights into the chemical communication of honeybee. Our results clearly show that, in this behavioural context (using the PER paradigm), honeybees are able to discriminate between cuticular waxes of drones and workers of different tasks and that it is not the aliphatic hydrocarbons, but the esters and polar components in the cuticular waxes, that are providing the discriminative cues for the insects.

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Résumé – Les abeilles domestiques distinguent les cires cuticulaires d'après les esters et les composés polaires. La composition chimique des cires d'insectes joue un rôle important dans la communication chimique des insectes sociaux en tant que signal pour la reconnaissance des individus apparentés ou des membres de la colonie. La plupart des recherches antérieures sur la capacité des insectes à distinguer différentes cires se sont intéressées principalement aux hydrocarbures aliphatiques (Howard, 1993 ; Smith and Breed, 1995), bien que très peu d'études aient montré que les abeilles réagissaient aux hydrocarbures (Breed, 1998 ; Lahav, 1999). Néanmoins on suppose que les abeilles domestiques (*Apis mellifera* L.) peuvent distinguer les sexes et les castes d'après les différences de composition chimique des cires cuticulaires (Fröhlich et al., 2000b). L'objectif de ce travail était de savoir si les abeilles sont capables de distinguer les cires des mâles des cires des ouvrières réalisant différentes tâches et de déterminer les classes de substances qui fournissent aux abeilles les signaux discriminatifs.
Des mâles et des ouvrières d'abeilles occupés à diverses tâches (butineuses, stockeuses de nourriture, abeilles accompagnatrices) ont été prélevés dans une même colonie et tués par l'azote liquide. Les cires ont été

extraites en plongeant les abeilles dans le chloroforme ($CHCl_3$) durant 30 s environ, à température ambiante. Pour les mâles et les ouvrières stockeuses de nourriture, les extraits totaux de cire ont été séparés en 5 fractions par une méthode d'extraction de phase solide décrite précédemment (Nass et al. 1998) : fraction A (éluée à l'hexane), fraction B (éluée après la fraction A au diéthyléther), fraction C (éluée après la fraction A au chlorure d'isopropyle), fraction D (éluée après la fraction C au diéthyléther), fraction E (éluée après la fraction D par un mélange de $CHCl_3$, de méthanol et d'eau dans les proportions 13:5:1). La fraction B renferme donc les mêmes substances que les fractions C et D réunies.

Les analyses chimiques quantitatives par chromatographie en phase gazeuse et spectrométrie de masse ont montré des différences significatives dans la composition chimique des cires cuticulaires des mâles et des différentes catégories d'ouvrières (Tabs. II et III). La fraction A (32–35 % de la masse totale des cires cuticulaires) consiste en hydrocarbures aliphatiques saturés et insaturés, ramifiés et non ramifiés. La fraction C (21–30 % de la masse totale) renferme des esters d'alkyle et des esters d'alkyle insaturés, la fraction D (2–4 %) renferme des esters d'alkyle hydroxylés, des alcools primaires et des acides. La fraction E (31–43 %) comporte des composés très polaires qui ne peuvent être analysés par chromatographie en phase gazeuse. Les analyses discriminantes ont montré des différences significatives dans la composition chimique des cires.

Le conditionnement discriminatif du réflexe d'extension du proboscis (PER) a été fait selon la méthode précédemment publiée (Fröhlich et al., 2000b). Pour cela des mâles et des ouvrières de différentes catégories de tâches ont été conditionnés aux extraits totaux et aux fractions de cire. Nos résultats montrent que les abeilles distinguent significativement les extraits cuticulaires des mâles de ceux des stockeuses de nourriture, des butineuses et des accompagnatrices (Tabs. IV et V).

Les résultats montrent clairement que, dans ce contexte comportemental utilisant le paradigme du PER, les abeilles sont capables de distinguer les cires cuticulaires des mâles de celles des différentes classes d'ouvrières et que ce ne sont pas les hydrocarbures aliphatiques, comme on le pensait jusqu'à présent, mais les esters, les esters insaturés, les alcools primaires et les acides (fraction C et D) qui fournissent les signaux discriminatifs (Tab. IV, Fig. 1).

***Apis mellifera* / cire cuticulaire / composition chimique / réflexe extension proboscis / conditionnement discriminatif**

Zusammenfassung – Honigbienen unterscheiden Kutikularwachse anhand von Estern und polaren Komponenten. Die chemische Zusammensetzung der Kutikularwachse von sozialen Insekten spielt eine groβe Rolle bei der chemischen Kommunikation im Insektenstaat, sowohl bei der Verwandten-, als auch bei der Nestgenossenerkennung. Die meisten bisherigen Studien zur Unterscheidungsfähigkeit von Wachsen durch Insekten haben sich hauptsächlich auf die aliphatischen Kohlenwasserstoffe konzentriert (Howard, 1993; Smith und Breed, 1995), obwohl nur in wenigen Studien die direkte Erkennung von Aliphaten durch Insekten nachgewiesen ist (Breed, 1998; Lahav et al., 1999). Weiterhin wird angenommen, dass Honigbienen auf der Basis von chemischen Unterschieden in der Kutikularwachszusammensetzung zwischen verschiedenen Kasten und Geschlechtern unterscheiden können (Fröhlich et al., 2000b). Die vorliegende Studie sollte prüfen, ob Honigbienen in der Lage sind, zwischen Kutikularwachsen von Drohnen und Arbeiterinnen verschiedener Berufsgruppen zu unterscheiden. Zusätzlich sollte geklärt werden, in welchen Substanzklassen die Erkennungsschlüssel für die Honigbienen liegen. Drohnen und Arbeiterinnen verschiedener Berufsgruppen (Sammlerinnen, Innendienstbienen und Hofstaatbienen) von *Apis*

mellifera L. wurden aus einem Stock abgefangen und in flüssigem Stickstoff getötet. Die Kutikulawachse wurden durch Waschung der Bienen mit Chloroform bei Raumtemperatur für ca. 30 s gewonnen. Die Gesamtextrakte der Kutikularwachse von Drohnen und Innendienstbienen wurden mit Hilfe einer Festphasenextraktion (Nass et al., 1998) in folgende Fraktionen getrennt: Fraktion A (mit Hexan eluiert), Fraktion B (nach Fraktion A mit Diethylether eluiert), Fraktion C (nach Fraktion A mit Isopropylchlorid eluiert), Fraktion D (nach Fraktion C mit Diethylether eluiert), Fraktion E (nach Fraktion D mit einer Mischung aus Chloroform, Methanol und Wasser 13:5:1 eluiert). Die Fraktion B bestand somit aus den gleichen Substanzen wie Fraktion C und D.

Die quantitativen Analysen mittels Gaschromatographie und Massenspektrometrie zeigten signifikante Unterschiede in der Zusammensetzung der Kutikularwachse von Drohnen und Arbeiterinnen verschiedener Berufsgruppen (Tab. II und III). Fraktion A (32–35 % der Gesamtmenge an Kutikularwachs) bestand aus ungesättigten und gesättigten, verzweigten und unverzweigten aliphatischen Kohlenwasserstoffen. Fraktion C enthielt Ester und ungesättigte Ester (21–30 % der Gesamtmenge an Kutikularwachs) und Fraktion D (nur 2–4 % der Gesamtmenge an Kutikularwachs) bestand aus Hydroxyestern, primären Alkoholen und Säuren. Fraktion E (31–43 % der Gesamtmenge an Kutikulawachs) enthielt sehr polare Substanzen, die nicht für die gaschromatographische Analytik geeignet waren. Mittels Diskriminantenfunktionsanalysen konnten die verschiedenen Kutikularwachse anhand ihrer chemischen Zusammensetzung signifikant unterschieden werden.

Um zu prüfen, inwieweit die Bienen die verschiedenen Wachse unterscheiden können, wurden Verhaltensversuche mit dem Rüsselstreckreflex der Bienen durchgeführt. Dazu wurden auf die Gesamtextrakte und die Fraktionen der Wachse von Drohnen und Arbeiterinnen verschiedener Berufsgruppen nach einer bewährten Methode (Fröhlich et al., 2000a) differentiell konditioniert. Es konnte gezeigt werden, dass Honigbienen in der Lage sind, zwischen den Gesamtextrakten der Drohnen und Arbeiterinnen unterschiedlicher Berufsgruppen signifikant zu unterscheiden (Tab. IV und V). Bei den Verhaltensversuchen mit den verschiedenen Fraktionen nutzten die Bienen die Ester, ungesättigten Ester, Säuren , Alkohole und Hydroxyester, um zwischen den Kutikularwachsen zu unterscheiden.

Die Ergebnisse der vorliegenden Studie zeigen deutlich, dass Honigbienen in diesem Verhaltenskontext (Rüsselstreckreflex) in der Lage sind, die verschiedenen Kutikularwachse von Drohnen und Arbeiterinnen unterschiedlicher Berufsgruppen zu diskriminieren. Dabei nutzen die Bienen nicht, wie weithin angenommen, die aliphatischen Kohlenwasserstoffe, sondern die Ester und polaren Substanzklassen der Wachse zur Unterscheidung.

***Apis mellifera* / Kutikularwachse / chemische Komponenten / Rüsselstreckreflex / Unterscheidung von Wachsen**

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