

ANALYSIS OF INDIVIDUAL OIL GLAND SECRETION PROFILES IN ORIBATID MITES (ACARI: ORIBATIDA)

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ABSTRACT - Oil glands are paired opisthosomal sac-like glands that characterize the so-called “glandulate Oribatida” (Parhyposomata, Mixonomata, Desmonomata, Brachypylina, Astigmata). Among glandulate groups, a novel pool of characters for phylogenetic analyses was recently introduced by investigations into the chemistry of oil gland secretions. Secretion profiles appear to be species-specific, and moreover, reveal a clear evolutionary trend from early-derivative glandulate groups to late-derivative brachypylines. Due to limited secretion volumes, gas chromatographic-mass spectrometric studies thus far have mainly relied on pooled extracts from multiple individuals. Herein we present an extended approach to analyse individual profiles, using two model species. In *Archezogetes longisetosus* Aoki (Trhypochthoniidae), derived from a laboratory culture, oil gland secretions were homogenous among individuals and yielded ca. 325ng of secretion per individual. In strong contrast, between zero and 7000ng of secretion was extracted from individuals of *Collohmanna gigantea* Sellnick (Collohmanniidae) collected from nature, and several distinct sub-profiles were detected. Such detailed data regarding intraspecific variation of secretion profiles both within and among populations are expected to serve as novel tools to study poorly-known population relationships in Oribatida.

Key words - Acari, Collohmanniidae, Trhypochthoniidae, chemosystematics, chemical ecology, opisthotal glands, *Archezogetes*, *Collohmanna*, Austria.

INTRODUCTION

In addition to traditional morphological data and accumulating molecular phylogenetic studies, chemical profiles from secretions of opisthotal (“oil”) glands have emerged as phylogenetically important characters among “glandulate” Oribatida (sensu Norton, 1998: i.e. Parhyposomata, Mixonomata, Desmonomata, Brachypylina, Astigmata). Oil glands are paired exocrine sac-like organs that are typically present in the posterior part of the hysterosoma. These glands have been shown to produce species-specific multicomponent secretions, i.e. certain chemical components as well as combinations of components in specific patterns that can clearly characterize oribatid groups at different taxonomic levels (Raspotnig, 2006b).

Whereas morphological and molecular investigations can be carried out on single individuals, the small size and limited secretion volumes of oribatid mites have required the pooling of secretion extracts for chemical

analyses in the majority of published studies. Profiles from pooled samples thus represent a “mean” and miss potential intraspecific variation. For example, about 10 individuals were used to prepare each studied oil gland extract of *Trhypochthonius tectorum* (Raspotnig *et al.*, 2004) and *Trhypochthoniellus crassus* (Sakata *et al.*, 1995). Similar studies of *Hermannia convexa* (Raspotnig *et al.*, 2005a), *Platynothrus peltifer* (Raspotnig *et al.*, 2005b) and *Schelorbates* ssp. (Takada *et al.*, 2005) required 4 to 20 individuals per extract. For *Collohmanna gigantea* (a large oribatid species with well-developed oil glands), Raspotnig *et al.* (2001) prepared extracts of about 20 individuals for each successful analysis. In these studies, the chemical identification of components in oil gland secretions was a main focus, and pooling ensured sufficient biological material. Other authors, such as Sakata and Norton (2001, 2003), Sakata *et al.* (2003) and Shimano *et al.* (2002), have successfully investigated extracts from individual mites, accepting the disadvantage of working with minute extract volumes of a few mi-

cro-litres, which was often sufficient for only a single injection into the analytical instrument. As the sensitivity of gas chromatographic-mass spectrometric instrumentation has improved, analysis of extracts of single individuals has become more feasible.

Herein, we report analyses and evaluation of individual secretion profiles in two model oribatid species, *Collohmanna gigantea* Sellnick, 1922 (Collohmanniidae) and *Archezogetes longisetosus* Aoki, 1965 (Trhypochthoniidae). Basic data on oil gland secretion profiles of these species have already been published (Raspotnig *et al.*, 2001; Sakata and Norton, 2003). We hope to promote further chemosystematic research in Oribatida by demonstrating improved analytical methods (thereby approaching the level of other systematic methods) and by introducing a novel tool to investigate population structure.

MATERIAL AND METHODS

Mites and mite extracts - Specimens of *Collohmanna gigantea* Sellnick, 1922 ("mixonomatan" Oribatida) were collected twice from the litter and fermentation layer of a beech- and hazel forest in Maria Rain (Carinthia, Austria): on June 15, 2007 (collection 1: 50 adult individuals) and August 26, 2007 (collection 2: 31 adult individuals). Individuals were collected by hand or with Berlese-Tullgren funnels. Specimens were handled with care to avoid release of their oil gland secretions. Extracts were prepared immediately after collection by submersing living, single individuals in hexane (1 individual per 50 µl) for a maximum of 30 min (secretions are directly discharged into the solvent: for details see e.g. Raspotnig *et al.*, 2001; 2004, 2005a, b). Crude extracts were used for analysis.

A laboratory strain of the asexual species *Archezogetes longisetosus* Aoki, 1965 ("desmonomatan" Oribatida) was provided by Dr. Michael Heethoff (University of Tuebingen, Germany). This strain was originally established by Dr. Roy A. Norton in 1993 from a single gravid individual from Puerto Rico (Heethoff *et al.* 2007a). Individuals were held in culture dishes for about six weeks and were fed on the periphyton from sycamore bark (*Platanus sp.*). Single individuals (n = 33) from culture dishes were carefully taken up by a brush and transferred into hexane (50 µl); extracts were prepared as described above.

Chemical analysis: gas chromatography-mass spectrometry - The analytical instruments for this study included a Trace gas chromatograph (GC) coupled to a Voyager mass spectrometer (MS) (both from Thermo, Vienna, Austria). The GC-column (a ZB-5MS fused silica capillary column: 30 m x 0.25 mm i.d., 0.25 µm film thickness from Phenomenex, Germany) was directly connected to the ion source of the MS. The splitless Grob injector was kept at 260°C, and helium (at a constant flow

rate of 1.5 ml/min) was used as a carrier gas. All data in the text refer to the following temperature program: initial temperature 50°C for 1 min, followed by an increase of 10°C/min to 200°C, with 15°C/min to 300°C, and an isothermal hold for 5 min. The ion source of the mass spectrometer and the transfer line were kept at 150°C and 310°C, respectively. Electron impact (EI) spectra were recorded at 70 eV.

Identification of compounds and authentic references - All compounds found in extracts are familiar to the authors and their identification on the basis of mass spectral data and comparison of retention times to authentic standards is described elsewhere (e.g. Raspotnig *et al.*, 2001, 2005b). Positions of double bonds in unsaturated hydrocarbons were not determined.

Quantitative data - Determination of relative compound abundance (in % peak area of whole secretion) was based on the integration of peak areas in the chromatograms. Absolute quantification was based on an external standard, neral, that is, an abundant component in the secretions of both species. Commercially available citral (neral content about 40%) was used to generate a neral calibration curve, covering a range from about 1 ng neral (per 2 µl and injection) up to 80 ng (per 2 µl and injection). The calibration curve was exponential and was described by the formula $y = 5.97 \times e^{x/185} - 4.72$ (regression coefficient: 0.99). Additionally, linear sub-curves were obtained for smaller ranges of concentration (e.g. 0.5-7.5 ng/2 µl of injection; 7.5-15 ng/2 µl of injection etc). Linear calibration curves were used especially for neral concentrations lower than 7.5 ng/2 µl, as the exponential curve offered poor resolution in this range. A neral concentration of 1.5 ng/2 µl was the lower limit of precise calculation, so lower amounts are referred to as "trace" (if still detectable) or as "-" (if not detected). Concentrations above 7.5 ng/2 µl were quantified using both non-linear and linear curves, taking the mean of both calculations (which were approximately the same). Absolute values of neral per individual were calculated based on a final extract volume of 40 µl (about 10 µl evaporated during extraction and transfer procedures). Absolute amounts of whole secretion per individual were calculated from the relative peak area of neral (thus leading to semi-quantitative data only).

RESULTS

Secretion profiles: qualitative analysis - Thirty-three individual extracts of *Archezogetes longisetosus* were analysed and all included the nine components shown by Sakata and Norton (2003), plus one additional compound. The compounds were identified as: 2,6-HMBD (= 2-hydroxy-6-methyl-benzaldehyde: peak a), neral (peak b), geranial (peak c), neryl formate (peak d), γ-acaridial (peak e), pentadecene (peak g), n-penta-

Table 1. Profiles of oil gland secretions and absolute amounts of neral (and whole secretion) in individuals of *Archeogozetes longisetosus*.

sample no.	relative amount ¹										absolute amount ²	
	2,6-HMBD	neral	geranial	neryl formate	γ -acaridial	C _{15:1}	C ₁₅	C _{17:2}	C _{17:1}	C ₁₇	neral	whole secretion
Ar-1	5.6	18.0	0.6	36.1	23.3	1.8	9.9	0.3	4.4	0.1	53	294
Ar-2	7.3	17.3	0.7	32.1	28.4	1.9	8.6	0.2	3.6	-	75	432
Ar-3	4.6	14.3	0.5	40.6	21.4	2.8	9.1	0.4	6.3	-	38	265
Ar-4	9.7	18.3	0.2	29.7	29.5	1.2	7.2	0.3	3.9	0.1	58	318
Ar-5	5.1	18.0	0.6	36.3	23.5	1.7	9.4	0.4	5.0	-	58	323
Ar-6	4.6	11.2	0.9	38.2	23.2	2.6	10.7	0.5	8.1	0.1	42	377
Ar-7	6.3	18.0	0.7	33.5	28.1	1.6	8.0	0.3	3.6	-	56	309
Ar-8	3.1	13.6	0.7	40.4	20.1	2.3	12.3	0.4	6.9	0.2	47	344
Ar-9	5.2	18.3	0.8	32.7	23.4	2.2	10.8	0.4	5.6	0.1	67	366
Ar-10	7.2	20.6	1.0	26.7	27.4	2.0	8.5	0.5	6.0	0.1	65	317
Ar-11	3.2	15.1	0.6	35.5	26.8	2.2	12.0	0.2	4.3	0.2	53	351
Ar-12	3.7	12.9	0.5	31.3	30.2	3.0	13.7	0.3	4.3	0.1	57	445
Ar-13	4.7	16.8	0.6	36.4	26.1	2.0	7.5	0.6	5.2	0.1	44	261
Ar-14	3.9	15.9	0.4	34.2	27.5	1.7	11.1	0.4	4.9	trace	53	331
Ar-15	1.9	10.0	1.0	43.6	19.3	3.3	12.9	0.6	7.3	0.2	30	305
Ar-16	7.4	16.3	0.7	30.3	31.4	1.4	8.4	0.3	3.9	0.1	52	321
Ar-17	3.2	13.0	0.7	41.5	23.0	2.2	10.0	0.4	5.9	0.2	38	289
Ar-18	5.3	14.5	0.6	39.0	26.9	1.5	8.2	0.3	3.6	0.1	49	339
Ar-19	4.9	18.8	0.8	27.8	27.9	2.2	11.0	0.6	5.8	0.1	51	268
Ar-20	2.4	11.6	0.9	41.8	20.0	2.9	12.4	0.7	7.4	0.1	33	286
Ar-21	2.9	14.9	0.6	35.8	28.4	2.0	10.8	0.3	4.4	0.1	49	331
Ar-22	2.3	21.8	0.5	41.0	10.3	2.5	14.0	0.8	6.5	0.3	36	164
Ar-23	3.4	15.0	0.5	38.2	27.0	1.8	9.5	0.3	4.2	0.1	45	297
Ar-24	4.0	16.7	0.6	32.7	30.0	1.7	9.4	0.3	4.5	0.1	72	430
Ar-25	3.6	19.8	0.6	32.9	26.5	1.5	6.1	0.6	8.1	0.2	44	220
Ar-26	4.0	16.3	0.5	28.1	31.0	2.0	10.8	0.4	7.0	0.1	75	462
Ar-27	6.7	16.7	0.9	28.3	33.0	1.5	8.7	0.3	3.9	0.1	70	419
Ar-28	-	15.8	-	35.5	14.5	-	17.1	-	17.1	-	26	163
Ar-29	3.9	17.1	0.8	31.9	29.4	1.8	10.3	0.3	4.5	0.1	68	394
Ar-30	1.9	20.0	1.0	32.3	20.5	2.6	14.4	0.5	6.6	0.2	56	282
Ar-31	4.3	15.2	0.4	35.5	25.8	2.1	10.0	0.5	5.6	0.1	43	282
Ar-32	3.7	13.9	0.6	32.2	30.7	2.0	11.0	0.4	5.6	0.2	52	377
Ar-33	4.7	14.4	0.5	35.8	26.6	2.0	9.9	0.4	5.5	0.2	53	366

¹ Relative abundance of components is given in % peak area of whole secretion.

² Absolute amounts of secretion (in nanograms) were calculated based on relative content of neral in the secretion (absolute neral content measured by an external synthetic standard).

Abbreviations: C₁₅ (pentadecane), C_{15:1} (pentadecene), C₁₇ (heptadecane), C_{17:1} (heptadecene), C_{17:2} (heptadecadiene).

Table 2. Profiles of oil gland secretions and absolute amounts of neral (and whole secretion) in individuals of *Collohimania gigantea* (collection # 1).

sample no.	relative amount ¹						absolute amount ²		type of profile
	2,6-HMBD	neral	geranial	neryl formate	C ₁₃ + acaridial	C ₁₅	neral	whole secretion	
I/Col-1	12.0	46.0	1.0	2.4	37.2	1.5	63	136	A
I/Col-2	0.2	38.1	0.5	trace	58.1	3.2	41	109	B3
I/Col-3	1.7	51.1	trace	trace	44.2	3.0	33	64	B1
I/Col-4	0.7	42.3	0.7	trace	51.4	4.9	33	78	B1
I/Col-5	-	43.8	-	-	50.0	6.3	trace	n.c.	D
I/Col-6	1.3	61.6	0.3	0.1	36.3	0.4	69	112	B2
I/Col-7	-	-	-	-	-	-	-	-	D
I/Col-8	-	trace	-	-	-	-	trace	trace	D
I/Col-9	3.8	36.1	0.5	0.5	58.4	0.7	39	108	B3
I/Col-10	1.4	44.5	0.5	0.2	52.4	0.9	73	164	B1
I/Col-11	4.0	50.2	0.4	0.4	43.2	1.8	41	81	B1
I/Col-12	trace	41.0	0.4	0.4	56.8	1.3	31	76	B3
I/Col-13	0.8	54.6	0.8	trace	42.2	1.7	29	54	B2
I/Col-14	trace	52.4	0.3	0.1	46.8	0.4	55	104	B1
I/Col-15	10.0	42.2	0.8	2.2	43.2	1.7	390	924	A
I/Col-16	0.9	55.7	0.5	-	42.1	0.9	33	60	B2
I/Col-17	-	66.2	1.4	-	31.0	1.4	28	42	B2
I/Col-18	6.0	43.8	0.7	0.5	42.6	6.4	593	1355	A
I/Col-19	-	30.9	-	-	69.1	-	29	95	B3
I/Col-20	-	trace	-	-	-	-	trace	trace	D
I/Col-21	0.7	47.6	0.2	0.3	50.6	0.6	60	125	B1
I/Col-22	0.8	45.0	0.5	0.1	53.1	0.6	59	132	B1
I/Col-23	2.8	53.4	0.9	0.1	39.6	3.3	648	1214	A
I/Col-24	-	30.7	-	-	69.3	-	trace	n.c.	D
I/Col-25	1.1	64.2	1.1	1.1	29.5	3.2	29	45	B2
I/Col-26	1.3	37.5	2.5	-	56.3	2.5	trace	n.c.	D
I/Col-27	0.6	46.8	0.5	-	51.2	0.9	129	275	B1
I/Col-28	0.4	41.0	0.4	0.2	56.0	2.0	39	95	B3
I/Col-29	1.3	47.7	0.2	-	48.9	1.9	48	100	B1
I/Col-30	-	78.3	-	-	21.7	-	29	36	B2
I/Col-31	0.7	43.1	0.7	-	53.5	2.1	29	68	B3
I/Col-32	-	trace	-	-	-	-	trace	trace	D
I/Col-33	-	34.1	0.7	-	63.0	2.2	28	82	B3
I/Col-34	0.7	47.8	0.5	0.1	50.3	0.7	95	199	B1
I/Col-35	-	34.2	-	-	65.9	-	trace	n.c.	D
I/Col-36	1.1	47.4	0.5	0.1	47.0	4.1	182	385	B1
I/Col-37	0.2	63.6	0.2	-	34.6	1.4	70	110	B2
I/Col-38	0.6	41.1	0.4	0.1	57.1	0.7	49	120	B3
I/Col-39	-	76.9	-	-	23.1	-	trace	n.c.	D
I/Col-40	-	-	-	-	-	-	-	-	D
I/Col-41	0.6	31.1	0.6	-	65.9	1.8	28	91	B3
I/Col-42	-	trace	-	-	-	-	trace	trace	D
I/Col-43	1.4	67.1	1.4	-	27.4	2.7	28	42	B2
I/Col-44	-	51.7	-	-	48.3	-	trace	n.c.	D
I/Col-45	0.3	47.2	0.4	-	51.2	0.9	114	241	B1
I/Col-46	-	18.2	-	-	81.8	-	trace	n.c.	D
I/Col-47	3.0	47.6	0.5	0.8	47.6	0.6	82	173	B1
I/Col-48	-	20.0	-	-	80.0	-	trace	n.c.	D
I/Col-49	-	trace	-	-	-	-	trace	trace	D
I/Col-50	-	53.2	-	-	46.8	-	trace	n.c.	D

¹ Relative abundance of components is given in % peak area of whole secretion. Tridecane (C₁₃) and γ -acaridial could not be chromatographically separated. Samples and profiles in bold letters indicate large absolute amounts of secretion.

² Absolute amounts of secretions were calculated based on measured amounts of neral; calculations, in case of low or trace amounts were not always possible (= n.c.: not calculated).

Abbreviations: C₁₃ (tridecane), C₁₅ (pentadecane).

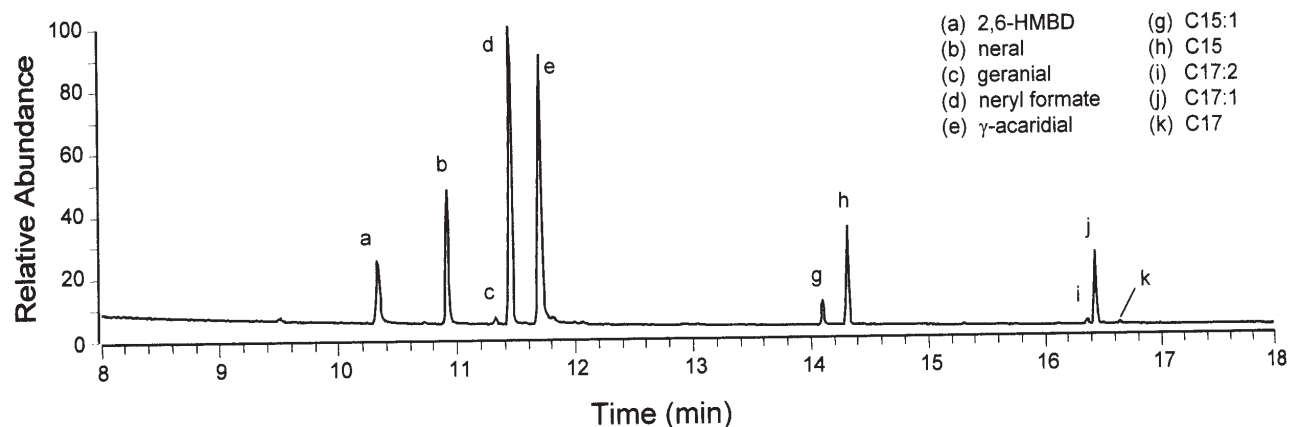


Fig. 1. Typical gas chromatographic pattern of components, corresponding to the oil gland secretion profile, found in extracts of individual *Archeogozetes longisetosus*.

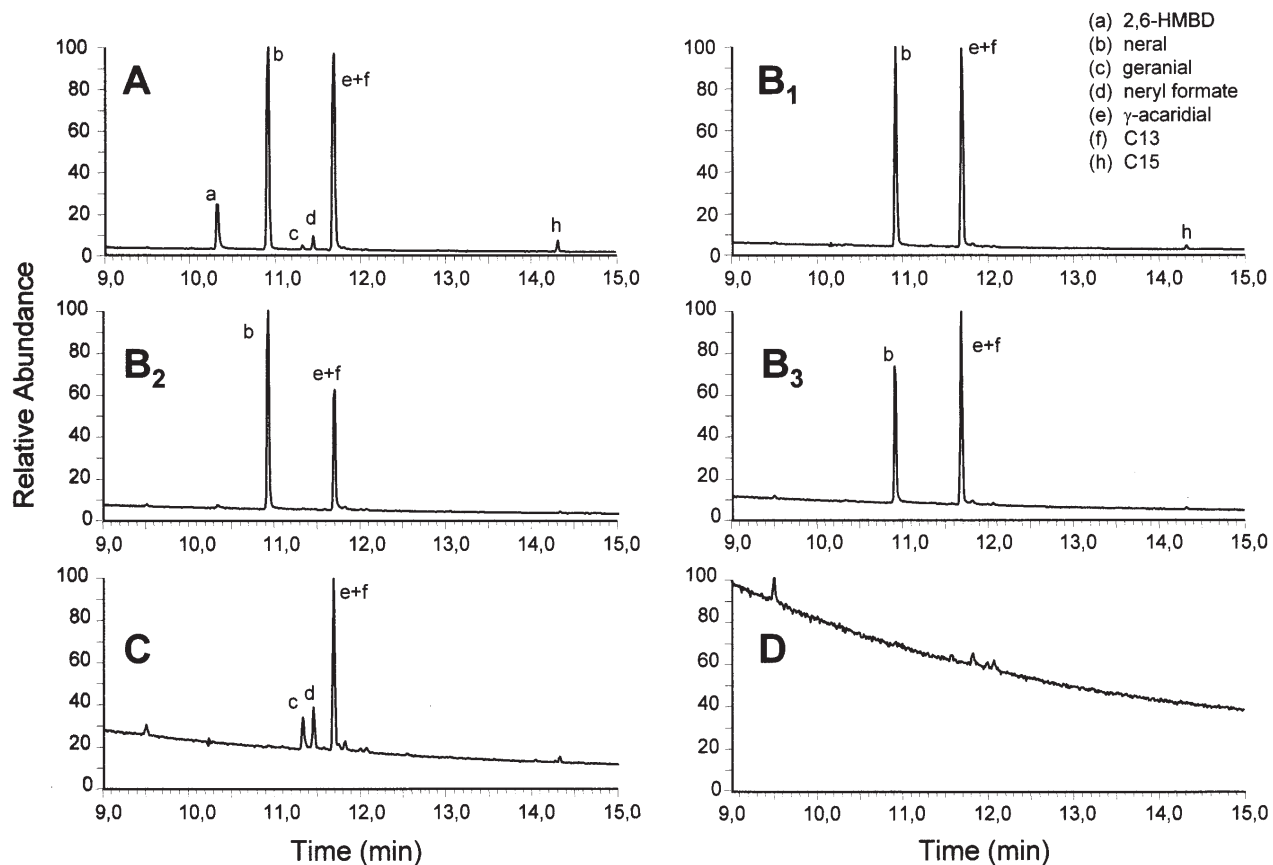


Fig. 2. Different types of oil gland secretion profiles found in individuals of *Collohmanna gigantea*. A. Full profile, showing all seven oil gland components in their typical relative abundance (see Raspotnig, 2006a and text). B₁-B₃. Reduced profiles, showing neral and tridecane (plus γ -acaridial*) only. Sub-profile B₁: equal amounts of neral and tridecane (plus γ -acaridial*). Sub-profile B₂: amount of neral > tridecane (plus γ -acaridial*). Sub-profile B₃: amount of neral < tridecane (plus γ -acaridial*). C. Aberrant profile, showing geranial, neryl formate and tridecane (plus γ -acaridial*). D. Completely reduced profile, no components detectable. For details, see text. *Tridecane and γ -acaridial were not separable under the given chromatographic conditions and eluted within one peak (= peak e+f).

Table 3. Profiles of oil gland secretions and absolute amounts of neral (and whole secretion) in individuals of *Collohmanna gigantea* (collection # 2).

sample no.	relative amount ¹						absolute amount ²		type of profile
	2,6-HMBD	neral	geranial	neryl formate	C ₁₃ + acaridial	C ₁₅	neral	whole secretion	
II/Col-1 (♀)	-	-	-	-	-	-	-	-	D
II/Col-2 (♀)	0.1	0.6	15.6	17.0	64.4	2.3	trace	n.c.	C
II/Col-3 (♀)	2.8	36.8	0.5	0.8	58.2	1.1	42	113	B3
II/Col-4 (♂)	-	43.6	-	-	51.3	5.1	trace	n.c.	D
II/Col-5 (♀)	-	17.5	-	-	81.0	1.5	trace	n.c.	D
II/Col-6 (♂)	0.2	25.1	0.4	1.0	71.2	2.2	33	134	B3
II/Col-7 (♀)	-	trace	-	-	trace	-	trace	n.c.	D
II/Col-8 (♂)	1.7	46.3	0.3	0.6	49.4	1.7	176	381	B1
II/Col-9 (♀)	0.8	36.7	0.3	0.3	58.9	3.1	35	94	B3
II/Col-10 (♂)	-	-	-	11.4	88.6	-	-	n.c.	C
II/Col-11 (♀)	16.8	22.2	4.0	17.2	35.0	4.8	1566	7065	A
II/Col-12 (♀)	5.1	28.7	1.4	9.2	53.5	2.1	46	160	A
II/Col-13 (♀)	1.9	38.4	0.9	0.4	56.9	1.5	58	151	B3
II/Col-14 (♂)	1.1	46.8	0.6	0.2	49.0	2.3	142	303	B1
II/Col-15 (♀)	-	-	-	trace	trace	-	trace	trace	D
II/Col-16 (♀)	0.8	2.1	11.0	19.3	65.7	1.1	-	n.c.	C
II/Col-17 (♀)	0.1	31.3	0.4	0.4	62.3	5.3	40	127	B3
II/Col-18 (♀)	2.4	12.3	0.3	6.9	75.0	3.1	trace	n.c.	C
II/Col-19 (♀)	-	-	-	-	trace	-	-	trace	D
II/Col-20 (♂)	3.7	37.2	0.7	3.9	52.6	1.9	103	277	A
II/Col-21 (♂)	-	31.7	-	-	67.5	0.8	43	134	B3
II/Col-22 (♂)	1.4	46.2	0.4	0.3	48.9	2.8	165	358	B1
II/Col-23 (♂)	0.7	43.5	0.5	0.1	52.6	2.6	127	292	B1
II/Col-24 (♂)	-	15.8	-	-	80.0	4.2	trace	n.c.	D
II/Col-25 (♂)	-	23.5	-	-	73.8	2.7	trace	n.c.	D
II/Col-26 (♀)	10.5	46.9	1.4	2.0	35.0	4.2	540	1151	A
II/Col-27 (♀)	4.7	57.1	0.7	0.3	35.8	1.5	144	252	B2
II/Col-28 (♂)	-	27.0	-	-	70.2	2.8	31	116	B3
II/Col-29 (♂)	0.1	38.4	0.4	-	56.9	4.2	101	263	B3
II/Col-30 (♀)	-	trace	5.1	19.0	71.2	4.7	trace	n.c.	C
II/Col-31 (♀)	7.3	42.6	1.1	2.4	43.4	3.2	348	818	A

¹ Relative abundance of components is given in % peak area of whole secretion. Tridecane (C₁₃) and γ -acariadial could not be chromatographically separated. Samples and profiles in bold letters indicate large absolute amounts of secretion.

² Absolute amounts of secretions were calculated based on measured amounts of neral; calculations, in case of low or trace amounts were not always possible (= n.c.: not calculated).

Abbreviations: C₁₃ (tridecane), C₁₅ (pentadecane).

decane (peak h), heptadecadiene (peak i), heptadecene (peak j). The 10th component, heptadecane (peak k), was detected in trace quantities only.

In extracts of *Collohmanna gigantea*, a maximum of seven components (consistent with Raspotnig, 2006b) were found: 2,6-HMBD (peak a), neral (peak b), geranial (peak c), neryl formate (peak d), γ -acariadial and n-tridecane (both eluted - not separable - at the same retention time in a peak e+f which showed a mixed mass spectrum), and n-pentadecane (peak h).

Inter-individual variations of secretion profiles -

Relative compound abundances in individual secretion profiles are shown in Tables 1-3. In *Archezogozetes longisetosus*, individual profiles varied little among the 33 ex-

tracts investigated (Fig. 1). The average *Archezogozetes*-profile included: 2,6-HMBD (4.4 ± 1.9%), neral (16.1 ± 2.7%), geranial (0.6 ± 0.2%), neryl formate (= main component: 34.8 ± 4.4%), γ -acariadial (25.5 ± 4.9%), C₁₅:1 (2.0 ± 0.6%), C₁₅ (10.4 ± 2.3%), C₁₇:2 (0.4 ± 0.2%), C₁₇:1 (5.7 ± 2.4%), C₁₇ (0.1 ± 0.1%). The average absolute amount of neral was 52 ± 13 ng, leading to a calculated 325 ± 71 ng of whole secretion per individual.

In *Collohmanna gigantea*, at least four distinct profiles were present (Fig. 2A-D). Profile A, which included all known components in the relative abundance that was described previously (see Raspotnig, 2006a), was seen in only 8% of individuals from collection I and about 16% of individuals from collection 2. This profile, even though

neral and tridecane (together with γ -acariidal) were the main components, was characterised by an at least 10%-portion of the remaining minor components (i.e. neral plus tridecane < 90% of secretion).

The most common profile, B, found in 60% of individuals of collection 1 and about 42% of individuals of collection 2, consisted almost exclusively of neral and tridecane (together with γ -acariidal), which amounted to more than 90% of the secretion. Other components were not detectable or were present in trace amounts. Furthermore, profile B showed three subprofiles (Fig. 2, profiles B₁-B₃): profile B₁ with the amount of neral equal (=within a range of 10%) to that of tridecane (again together with γ -acariidal); profile B₂ with the amount of neral higher than that of tridecane (and γ -acariidal); and profile B₃ with the amount of neral less than that of tridecane (and γ -acariidal). In collections 1 and 2, respectively: profile B₁ was found in about 26% and 13% of individuals; profile B₂ was found in about 16% and 3% of individuals; and profile B₃ was found in about 18% and 26% of individuals.

In profile C (Fig. 2C), found in 13% of individuals from collection 2, tridecane (together with γ -acariidal) was the main component, with smaller amounts of geranial and neryl formate evident. This profile was not detected in any individuals of collection 1.

Profile D (Fig. 2D) is a completely reduced profile, found in about 32% and 26% of individuals from collections 1 and 2, respectively. The mites either contained no extractable secretion or secretion components were present in trace amounts only.

The overall profiles of both collections turned out to be similar. Mean values were: Collection 1: 2,6-HMBD ($1.2 \pm 2.4\%$) neral ($40.3 \pm 20.3\%$), geranial ($0.4 \pm 0.5\%$), neryl formate ($0.2 \pm 0.5\%$), tridecane plus γ -acariidal ($42.5 \pm 21.3\%$), pentadecane ($1.4 \pm 1.6\%$). Collection 2: 2,6-HMBD ($2.0 \pm 3.7\%$), neral ($25.8 \pm 18.2\%$), geranial ($1.5 \pm 3.4\%$), neryl formate ($3.6 \pm 6.3\%$), tridecane plus γ -acariidal ($51.9 \pm 24.2\%$), pentadecane ($2.3 \pm 1.6\%$).

Extremely large differences in absolute amounts of secretion were detected among individuals, ranging from no secretion at all to maximally 1566ng neral and (calculated) 7065ng of whole secretion, found in one individual of collection 2. On average, 67 ± 130 ng neral (corresponding to calculated 142 ± 276 ng secretion) was found in representatives of collection 1, and 121 ± 292 ng neral (i.e., 393 ± 1264 ng of whole secretion) was calculated for individuals of collection 2. Interestingly, profile A was typically found in individuals that contained large amounts of secretion (Tables 2 and 3).

DISCUSSION

Intraspecific stability and variability of oil gland secretion profiles - So far, oil gland secretion profiles of oribatids have been considered highly species-specific (or

at least restricted to small groups of species), and were shown to exhibit little variation, irrespective of population, sample locality or seasonal influences (e.g. Raspotnig *et al.*, 2001; 2005b). This profile stability was considered a base solid enough to evaluate chemical data of profiles for chemosystematics (e.g. Raspotnig, 2006b). While individual extracts of *Archezogozetes longisetosus* exhibited a consistent, stable profile together with similar absolute amounts of secretion per individual, *Collohmanna gigantea* did not. Following several studies supporting a stable secretion profile in this species (e.g. Raspotnig, 2006a; Raspotnig *et al.*, 2001) our results were rather surprising, but can be explained.

Archezogozetes longisetosus is a parthenogenetic species; all individuals studied originated from the same (clonal) laboratory culture and were reared under the same conditions. Low variation in secretion profiles may reflect the genetic homogeneity of the population and an equal physiological status of individuals. By contrast, *C. gigantea* is a bisexual species, although no sex-related differences in secretion profiles were noticed: the four distinct profiles were equally distributed among males and females (Table 3). But *C. gigantea* produces an alarm pheromone in its oil glands (Raspotnig, 2006a), and when disturbed, exudates are readily emitted, thus reducing the amount of remaining, extractable secretion in these individuals. Even though great care was taken to treat individuals gently in the course of collection from soil, exposure to a certain degree of disturbance could not be avoided. We believe that differences in treatment during collection were sufficient to induce secretion emission to a degree that it showed variation among individuals, which was reflected in the heterogeneity of measurable secretion among individual extracts. We assume that the differences in secretion profiles were related to this variation, as is especially evident when comparing the full profile A (which was found in obviously undisturbed individuals that contained much secretion) and profile D (= no secretion in highly-disturbed individuals). Profile B, which was found in the majority of individual extracts, most likely represents a reduced profile of moderately-disturbed individuals; it shows only the main components of secretion. This profile was consistently found in specimens containing a rather low absolute amount (about 50-300ng) of secretion. In these cases, minor components - 2,6-HMBD, geranial, neryl formate and pentadecane - were only detectable in traces or were below the limit of detection.

A further factor leading to heterogeneity in secretion profiles relates to a methodological problem: due to exponential relations between peak area and amount of components (see Materials and Methods), the relative abundance of compounds may be distorted between extracts with extremely high or low absolute amounts of secretion. In contrast, profile C, found for the first time in *C. gigantea*, may indicate a state of refilling of glandular res-

ervoirs, thus distorting proportions of secretion components during secretion production. Data from functional morphology of oil glands might support this idea. In fact, different secretory areas were reported to be present around the oil gland reservoirs of *C. gigantea* (Raspotnig *et al.*, 2003), each possibly responsible for the production of a distinct part of the chemically diverse secretion.

Absolute amounts of oil gland secretions - For oribatids, absolute amounts of oil gland secretions per individual are only known for deutonymphs of *Nothrus palustris*, which contain about 100ng geranial as the major component of their oil gland secretion (Shimano *et al.*, 2002). With an average individual weight of 100µg (see Heethoff and Koerner, 2007), *Archezogozetes longisetosus* produces about 300ng of secretion which amounts to about 0.3% of the body mass. *Collohmanna gigantea* - being one of the largest oribatid species - obviously is capable of producing and storing much more secretion. According to our data, about 7 µg of secretion (1.5 µg of neral) per individual is the preliminary maximum. These data correspond to a recent behavioural study (Raspotnig, 2006a): when disturbed, even one individual of *C. gigantea* can emit such large amounts secretion that a lemon scent is detectable by humans. Raspotnig (2006a) reported that emission can be stimulated 5-6 times in one mite individual before reservoirs are exhausted. Each of these discharges induces strong alarm behaviour in neighbouring individuals, due to four pheromonally-active components in the secretion; these are 2,6-HMBD, neryl formate, neral and geranial. Strong alarm behaviour is elicited by about 1×10^{15} molecules of synthetic neral (corresponding to about 250 ng). Thus, a 6-fold emission, each emission assumed to contain neral in the described range (i.e., a total of about 1.5 µg), would be roughly consistent with the amount of secretion present in an individual with well-filled glandular reservoirs. All data on absolute amounts of secretion given in the present study must be regarded as approximations, since these were based on, and extrapolated from, measurements of neral-contents.

Profiles as tools for chemosystematics? - As already emphasised in previous studies, the evaluation of oil gland profiles for chemosystematic purposes requires extensive investigations using a large number of individuals. At least in some species, profiles are subject to a certain degree of variability. In *C. gigantea*, such extensive studies with several hundred individuals have already been performed (Raspotnig *et al.*, 2001; Raspotnig, 2006b). Interestingly, these investigations, which all relied on pooled extracts, showed a *Collohmanna*-profile that is fully consistent with profile A of the present study. This is because in pooled extracts, individuals that contain much secretion (profile A-individuals) mask the profiles from those with reduced amounts of secretion. As a result, profiles from pooled extracts of *C. gigantea* mainly

reflect the original profile, and thus are useful in chemosystematics.

Analysis of individual extracts offers a new potential source of data for obtaining insights into population variability. While this technique can currently be used only with rather large-sized oribatids having well-developed oil glands, it may help in the differentiation of syntopically-living and morphologically similar oribatid species of the same genus. For example, the latter phenomenon is currently being studied in syntopical *Oribotritia* species that are well distinguishable by their oil gland secretion profiles (unpublished data). In *Platynothrus peltifer*, an asexual species that is widely distributed in very heterogenous populations (Heethoff *et al.*, 2007b), the measurement of individual profiles may be an additional tool to trace population limits or, in case of overlapping populations, to sort individuals into defined populations.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Austrian Science Fund (FWF), project number P18486. We further thank Dr. Michael Heethoff, University of Tuebingen, Germany for providing a laboratory culture of *Archezogozetes longisetosus*.

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