Abh. Ber. Naturkundemus. Görlitz	Band 76 Heft 1	S. 43 – 50	2004

ISSN 0373-7586

Beitrag zum 4. Milbenkundlichen Kolloquium vom 26. bis 27. September 2003
im Zoologischen Institut und Museum der Ernst-Moritz-Arndt-Universität Greifswald

Citral in oil gland secretions of Oribatida (Acari) – a key component for phylogenetic analyses

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Abstract

In the present paper, we have investigated the occurrence and composition of oil gland-derived citral in a number of species of Oribatida, representing members of Mixonomata, Desmonomata and Brachypylida: in *Collohmannia gigantea*, a model species for early citral-possessing mixonomatan Oribatida, neral-rich citral is present (less than 10 % geranial). In *Platynothrus peltifer*, a member of desmonomatan Oribatida, the neral/geranial-ratio is well-balanced and exclusively in desmonomatan Nothridae, exemplarily shown for *Nothrus silvestris*, geranial-rich citral is occurring (less than 10 % neral). Citral is lacking in oil gland secretions of *Oribotritia berlesei* and *Euphthiracarus cribrarius* (both Mixonomata), in the desmonomatan *Hermannia gibba* and in *Hermanniella granulata* (Brachypylida).

These results reject the hypothesis of the general occurrence of geranial-rich citral in »astigmatid-compound«-possessing Oribatida and also reject the hypothesis of geranial-rich citral as the ancestral condition for oil gland secretions in general. Actually, geranial-rich citral more likely is a derived autapomorph character within Oribatida and might represent a synapomorphy of Nothridae and basal Astigmata.

Keywords: opisthonotal glands, astigmatid compounds, *Collohmannia*, *Platynothrus*, *Nothrus*

Zusammenfassung

Citral in Öldrüsensekreten von Oribatiden (Acari): Eine Schlüsselkomponente für phylogenetische Untersuchungen – In der vorliegenden Arbeit wurde das Vorkommen und die Zusammensetzung von Citral aus Öldrüsen bei einer Anzahl von Oribatidenarten untersucht, die die Gruppen Mixonomata, Desmonomata und Brachypylida repräsentieren: bei *Collohmannia gigantea*, einer Modellart für frühe Citral-besitzende Mixonomata, existiert Neral-reiches Citral (weniger als 10 % Geranial-Anteil). Bei *Platynothrus peltifer*, einer desmonomaten Oribatide, ist das Neral/Geranial-Verhältnis ausgewogen, und ausschließlich bei den desmonomaten Nothridae, hier exemplarisch gezeigt für *Nothrus silvestris*, kommt Geranial-reiches Citral vor (Neral-Anteil unter 10 %). Dagegen fehlt Citral in Öldrüsensekreten von *Oribotritia berlesei* und *Euphthiracarus cribrarius* (beide Mixonomata), ebenso bei *Hermannia gibba* (Desmonomata) und *Hermanniella granulata* (Brachypylida).

Diese Ergebnisse sprechen deutlich gegen die Hypothese des generellen Vorkommens von Geranial-reichem Citral bei »Astigmata-Komponenten«-besitzenden Oribatiden und gegen die Einstufung von Geranial-reichem Citral als Primitivmerkmal von Öldrüsensekreten im allgemeinen. Vielmehr scheint Geranial-reiches Citral ein abgeleitetes, apomorphes Merkmal von Oribatiden zu sein und stellt möglicherweise eine Synapomorphie von Nothriden mit basalen astigmaten Milben dar.

1. Introduction

The monoterpene aldehyde citral – a stereoisomeric mixture of geranial (trans-, E-isomer of citral) and neral (cis-, Z-isomer of citral) – is a widely distributed component in oil gland secretions of Astigmata and Oribatida. These glands, also called opisthonotal glands, not only constitute the largest exocrine system in both Oribatida and Astigmata, but also are regarded to be homologous (Norton 1998), being comparable in position and morphological structure (Howard et al. 1988, Raspotnig et al. 2003) as well as in their chemistry (Kuwahara 1991, Sakata et al. 1995, Raspotnig et al. 2001, Sakata & Norton 2001). For the taxonomist, the occurrence or absence of opisthonotal glands devides the Oribatida into »non-glandulate« primitive groups (Palaeosomata and Enarthronota) and »glandulate« Oribatida which – in sensu strictu – would also comprise the Astigmata, obviously having evolved from an oil-gland-possessing oribatid ancestor (Norton 1998). Citral belongs to the so-called »astigmatid compounds« (sensu Sakata & Norton 2001) which characterise the oil gland secretions of a proposed evolutionary lineage from certain mixonomatan Oribatida to Desmonomata and finally to astigmatid mites (Raspotnig et al. 2001, Sakata & Norton 2001). Furthermore, the composition of oil-gland-derived citral – being dominated by neral or by geranial – appears to be group-specific: in the majority of astigmatid mites (generally believed to represent the most derived members of this lineage) neral-rich citral is present (Shimano et al. 2002). By contrast, basal Astigmata – such as the Histiostomatidae – possess geranial-rich citral (Kuwahara et al. 1991) which also may be true for Oribatida (Shimano et al. 2002). This situation would imply the ancestral occurrence of geranial-rich citral in Oribatida and basal Astigmata and the presence of neral-rich citral in Astigmata as the derived condition; thus, the composition of citral arises as a key character for phylogenetic analyses. Even though four publications on the chemistry of oil gland secretions are meanwhile available (Sakata et al. 1995, Raspotnig et al. 2001, Sakata & Norton 2001, Shimano et al. 2002), the information on the composition of citral is very limited.

Thus, in the present paper, we have investigated the occurrence and composition of oil gland-derived citral in a number of oribatid species, representing members of Mixonomata, Desmonomata and Brachpylida.

2. Materials and Methods

Specimen collection – *Collohmannia gigantea* Sellnick, 1922 (»Mixonomata«, Collohmanniidae), *Oribotritia berlesei* (Michael, 1898) (»Mixonomata«, Euphthiracaridae), *Euphthiracarus cribrarius* (Berlese, 1904) (»Mixonomata«, Euphthiracaridae), *Platynothrus peltifer* (C. L. Koch, 1839) (»Desmonomata«, Camisiidae), *Nothrus silvestris* Nicolet, 1855 (»Desmonomata«, Nothridae), *Hermannia gibba* (C. L. Koch, 1839) (»Desmonomata«, Hermanniidae) and *Hermanniella granulata* (Nicolet, 1855) (»Brachypylida«, Hermanniellidae) were collected from soil of mixed forests in Carinthia and Styria (both Austria), respectively.

Extraction of oil gland secretions – For *O. berlesei*, *E. cribrarius*, *P. peltifer*, and *H. gibba* only adult individuals were used for extraction; for *C. gigantea* adults of both sexes and juveniles of all stages and for *N. silvestris* adults and nymphs were extracted. Different numbers of mite individuals (3 – 30; depending on size of individuals, momentary availability and momentary sensitivity of analysing instruments) were extracted in 100 µl of hexane each for a maximum of half an hour before removing the supernatant to give crude mite extracts. In all, 16 samples of *C. gigantea* extracts (from 121 individuals), 12 samples of *P. peltifer* (from 150 individuals), 4 samples of *N. silvestris* (from 64 individuals), 1 sample of *O. berlesei* (3 individuals), 1 sample of *E. cribrarius* (about 20 individuals) and 1 sample of *H. gibba* (about 15 individuals) were used for this investigation.

Chemical analysis: gas chromatography – mass spectrometry – To analyse the composition of citral, we used a TRACE gas chromatograph (GC) coupled to a MD 800 mass spectrometer (MS) from Thermo-Quest (Vienna, Austria). The GC-column (a ZB-5 30 m x 25 mm x 0.25 µm capillary column from Phenomenex) was directly connected to the ion source of the MS. We kept the splitless Grob injector at 260 °C and used helium as a carrier gas at a constant flow rate. The ion source (EI-source) was kept at 200 °C, the transfer line at 310 °C. To separate the isomers of citral (and also to obtain the rest of the oil gland secretion), we used the following temperature program: initial temperature 50 °C (for 1 min), with 10 °C/min to 200 °C, with 15 °C/min to 300 °C and an isothermal hold at 300 °C for 5 min. The relative composition of citral was calculated by integrating the peak areas of neral and geranial. An authentic sample of citral (as a reference compound: 40 % neral and 60 % geranial) was purchased from Aldrich (Vienna, Austria). Isomerically pure neral and geranial was synthesised according to Raspotnig et al. (2001).

3. Results

Chemical identification of neral and geranial – At the given gas chromatographic conditions, the aldehydes of citral appeared well separated at retention times of 10.06 min and 10.48 min, respectively.

Both components had EI-mass spectra with characteristic monoterpene-fragmentation patterns (Fig. 1), both showing intense ions at m/z 41 (base ion in the first component) and at m/z 69 (base ion in the second component and originating from allylic cleavage).

The first component (Fig. 1A) only in a few samples exhibited a molecular ion of very low intensity (at m/z 152); in other samples the fragments at m/z 134 ($M^+ - 18$: loss of water) and m/z 137 ($M^+ - 15$: loss of CH_3) appeared to be the ions of highest mass. A comparison of the spectrum to that of authentic neral revealed uppermost correspondence.

The second component (Fig. 1B) showed a very similar EI-spectrum but always exhibited a (relatively) more prominent molecular ion (at m/z 152) and also moderately differed in the relative intensity of other fragments. The spectrum appeared to be identical to that of geranial.

For final identification, self-synthesised isomerically pure neral and geranial were used for a comparison of retention times, demonstrating full correspondence.

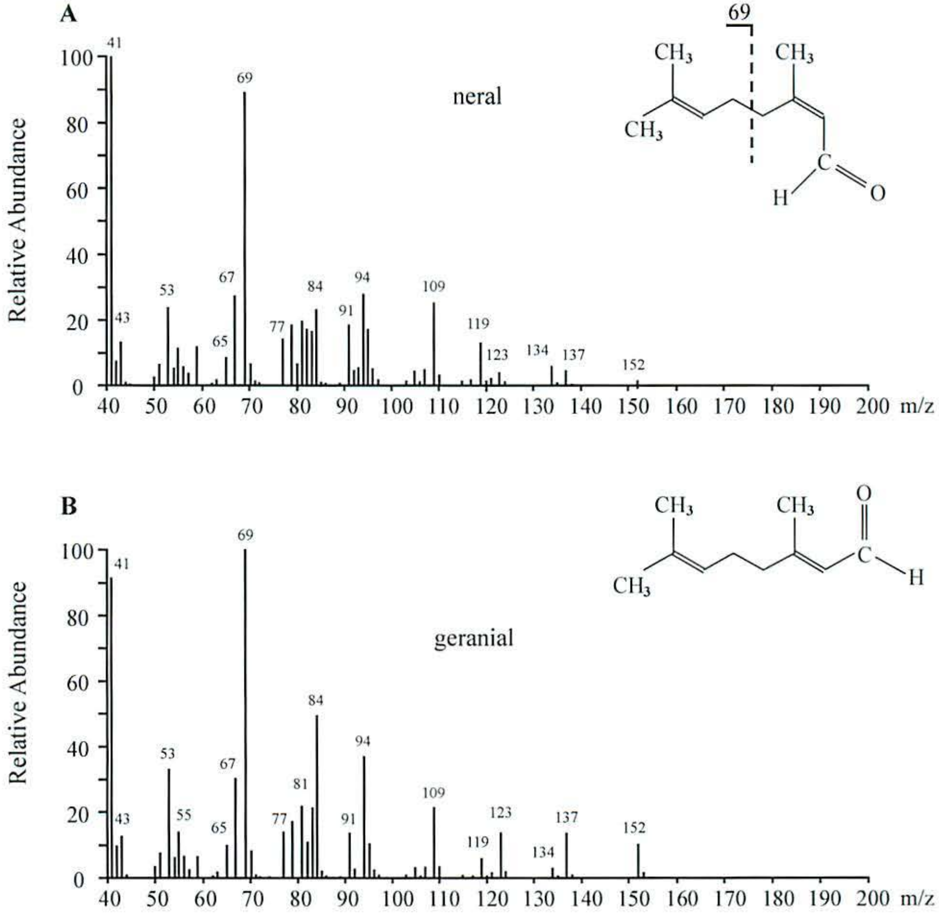

Fig. 1A, B Electron impact mass spectra of the stereoisomers of citral, neral and geranial (exemplarily recorded from the oil gland secretion of *Collohmannia gigantea*). Both components show similar fragmentation patterns, but differ in the relative intensity of certain fragment ions

Composition of citral in mite extracts – Citral was present in extracts of *C. gigantea*, *P. peltifer* and *N. palustris*, but not in extracts of *O. berlesei*, *E. cribrarius*, *H. gibba* and *H. granulata*.

Only in *P. peltifer*, the composition of citral was well-balanced, showing a neral : geranial-ratio of 43.24 : 56.76 (with a standard deviation of 8.34). In samples of *N. silvestris*, geranial-rich citral was present (geranial-content 97.88 %, standard deviation 1.05). No difference in the composition of citral was detected between samples of adults and samples of nymphs. Samples of *C. gigantea* clearly exhibited a third type of citral-composition, namely neral-rich citral with a neral-content on an avarage of 96.24 % (standard deviation: 2.37). Also in *C. gigantea*, males, females and nymphs showed no differences in their neral : geranial-ratios. For all three species, the neral : geranial-ratios of the samples investigated are summarised in Tab. 1 – 3.

Tab. 1 Composition of citral in *Platynothrus peltifer*

sample no. (number of individuals)	% neral (in citral)	% geranial (in citral)
Pl-A (20)	38.65	61.35
Pl-B (23)	54.60	45.40
Pl-C (30)	50.57	49.43
Pl-D (18)	27.15	72.85
Pl-E (15)	45.45	54.55
Pl-F (4)	32.95	67.05
Pl-G (11)	45.02	54.98
Pl-H (7)	43.31	56.69
Pl-I (3)	36.54	63.46
Pl-J (5)	41.77	58.23
Pl-K (4)	53.15	46.85
Pl-L (10)	49.75	50.25

Tab. 2 Composition of citral in *Nothrus silvestris*

sample no. (number of individuals)	% neral (in citral)	% geranial (in citral)
N-A (10)	0.85	99.15
N-B (22)	2.40	97.60
N-C (16)	3.37	96.63
N-D (16)	1.85	98.15

Tab. 3 Composition of citral in *Collohmannia gigantea*

sample no. (number of individuals)	% neral (in citral)	% geranial (in citral)
Co-A (10)	89.26	10.74
Co-B (13)	95.28	4.72
Co-C (11)	95.33	4.67
Co-D (11)	97.70	2.30
Co-E (4)	93.86	6.14
Co-F (6)	98.28	1.72
Co-G (10)	97.15	2.85
Co-H (5)	98.01	1.99
Co-I (3)	97.19	2.81
Co-J (13)	97.94	2.06
Co-K (4)	99.05	0.95
Co-L (5)	96.93	3.07
Co-M (6)	95.40	4.60
Co-N (7)	97.40	2.60
Co-O (7)	96.64	3.36
Co-P (6)	94.46	5.54

4. Discussion

According to our results, the hypothesis of geranial-rich citral as an ancestral oil gland-condition as specified in the introduction and the occurrence of geranial-dominated citral in Oribatida as discussed by Shimano et al. (2002) must be rejected. With regard to (the still very limited amount of) data on the oil gland chemistry in Oribatida, citral has obviously evolved first in middle-derivative mixonomatan oribatids: not yet being present in early-derivative mixonomatan Oribatida such as *Nehypochthonius porosus*, it can be found in mixonomatan Oribatida such as in *Perlohmannia* sp. in variable composition (Sakata & Norton 2001) and in *C. gigantea* in the form of neral-rich citral. [Neral in ethanolic extracts of *C. gigantea* appears to be very unstable and quickly isomerises to form geranial until a well-balanced neral : geranial-ratio is achieved; this fact hindered Raspotnig et al. (2001) to determine neral : geranial-ratios exactly in a previous investigation. This phenomenon is also known from neral-rich secretions of diverse Astigmata (Kizawa et al. 1993)]. Interestingly, neither *O. berlesei* nor *E. cribrarius* (both Mixonomata sensu Grandjean 1969) possess citral even though Euphthiracaroidea are supposed to represent close relatives to Collohmanniidae (sensu Haumann 1991). In both species, the lack of citral in oil gland secretions may be regarded as apomorphic, the more than there are some indications that basal members of Euphthiracaridae do synthesise astigmatid compounds (Norton, pers. comm.). In more-derived Oribatida such as in Desmonomata, citral is present in well-balanced composition in *Hydronothrus crispus* (= *Trhypochthoniellus crassus*: Trhypochthoniidae) (Sakata et al. 1995) and also in *Platynothrus peltifer* (Camisiidae) and possibly throughout the Crotonioidea (unpublished data). However, the remaining Desmonomata – even though being glandulate – appear to be no members of the lineage of astigmatid compounds-bearing Oribatida: as shown for the desmonomatan *H. gibba*, no citral is present (probably being true for all Hermannioidea and also for all Nanhermannioidea: unpublished observations; also *Hermanniella granulata*, a very basal brachypylid oribatid mite, has no citral). Furthermore, within the Crotonioidea, exclusively desmonomatan Nothridae appear to possess geranial-rich citral as it is the case in nymphs of *Nothrus palustris* (Shimano et al. 2002) and in adults and nymphs of *N. silvestris*.

In conclusion, Oribatida do not show uniformly composed citral: the neral-rich citral of *C. gigantea* may be regarded as an apomorphy within mixonomatan Oribatida as it is geranial-rich citral in Nothridae for Desmonomata. A well-balanced composition of citral may represent the general and most frequently realised state within citral-possessing Mixonomata and Desmonomata. However, the occurrence of geranial-rich citral in Nothridae may also be seen as a synapomorphy with basal Astigmata, confirming astigmatid mites' evolutionary origin within desmonomatan Oribatida.

5. Acknowledgements

This work was granted by the Austrian Science Foundation (Fonds zur Förderung der wissenschaftlichen Forschung – FWF), project number P14863.

6. References

Grandjean, F. (1969): Considerations sur le classement des Oribates: leur division en 6 groupes majeurs. – Acarologia **11**: 127 – 153

Haumann, G. (1991): Zur Phylogenie primitiver Oribatiden (Acari: Oribatida). – dbv-Verlag, Graz, 237 pp.

Howard, R. W., Y. Kuwahara, H. Suzuki & T. Suzuki (1988): Characterization of the hydrocarbons and external gland morphology of the opisthonotal glands of six species of mites (Acari: Astigmata). – Appl. Entomol. Zool. **23**: 58 – 66

Kizawa, Y., Y. Kuwahara, S. Matsuyama & T. Suzuki (1993): Chemical ecology of astigmatid mites XXXVI. Mite body catalyzes isomerization and reduction of neral (alarm pheromone component): common phenomenon? – J. Acarol. Soc. Jpn. **2**: 67 – 74

Kuwahara, Y. (1991): Pheromone study on astigmatid mites – alarm, aggregation and sex. – In Dusbabek, F. & V. Bukva (eds.): Modern Acarology, Vol. I. Academic Publishing, The Hague: 43 – 52

–, T. Satou & T. Suzuki (1991): Geranial as the alarm pheromone of *Histiostoma laboratorium* Hughes (Astigmata: Histiostomidae). – Appl. Entomol. Zool. **26**: 501 – 504

Norton, R. A. (1998): Morphological evidence for the evolutionary origin of Astigmata (Acari: Acariformes). – Exp. Appl. Acarol. **22**: 559 – 594

Raspotnig, G., R. Schuster, G. Krisper, G. Fauler & H. J. Leis (2001): Chemistry of the oil gland secretion of *Collohmannia gigantea* (Acari: Oribatida). – Exp. Appl. Acarol. **25**: 933 – 946

–, R. Schuster & G. Krisper (2003): Functional anatomy of oil glands in *Collohmannia gigantea* (Acari, Oribatida). – Zoomorphology **122**: 105 – 112

Sakata, T. & R. A. Norton (2001): Opisthonotal gland chemistry of early-derivative oribatid mites (Acari) and its relevance to systematic relationships of Astigmata. – Int. J. Acarol. **27**: 281 – 292

–, K. Tagami & Y. Kuwahara (1995): Chemical ecology of oribatid mites. I. Oil gland composition of *Hydronothrus crispus*. – J. Acarol. Soc. Jpn. **4**: 69 – 75

Shimano, S., T. Sakata, Y. Mizutani, Y. Kuwahara & J.-I. Aoki (2002): Geranial: the alarm pheromone in the nymphal stage of the oribatid mite, *Nothrus palustris*. – J. Chem. Ecol. **28**: 1831 – 1837

Manuscript accepted: 1 June 2004

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