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SHORT COMMUNICATION

Congruence of epicuticular hydrocarbons and tarsal secretions as a principle in beetles

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Abstract Within beetles, those species that are adapted to life on plants have developed widened tarsi with specialised hairy attachment structures. The capability to adhere to smooth surfaces is based on a liquid film on the surface of these structures, the composition of which is similar to the cuticular lipids. By means of a cluster analysis based on chemical similarities between samples obtained from tarsi or elytra of 35 species using solid phase microextraction, the present study strongly suggests that this chemical congruence is a principle in beetles. This supports the idea of tarsal liquids being part of the cuticular lipid layer and contributes to the understanding of liquid-mediated attachment systems.

Keywords Coleoptera · Cuticular hydrocarbons · Tarsal secretion · Cluster analysis · Adhesion · SPME

Introduction

Many insects use specialised tarsal adhesive structures to hold onto smooth surfaces in their environment. These structures are either smooth pads (e.g. in Orthoptera and Hymenoptera), soft pads with hairs (e.g. in Diptera) or brush-like hairy devices (e.g. in Coleoptera; Beutel and Gorb 2001, 2006), and they are most frequently found in plant-inhabiting species. All types of adhesive organs require a thin film of liquid to generate attachment forces by compensating nanoscale surface irregularities (Drechsler and Federle 2006; McFarlane and Tabor 1950). Forces are suggested to be based on physicochemical properties of the liquid film and its interaction with the wetted surfaces (Betz and Kölsch 2004; Drechsler and Federle 2006; Federle et al. 2002), and therefore should depend on the chemical composition of the liquid.

Previous investigators analysed tarsal liquids of several insect species deposited as footprints on glass (Attygalle et al. 2000; Federle et al. 2002; Ishii 1987; Kosaki and Yamaoka 1996; Vötsch et al. 2002) to study the composition of adhesive secretions. The suggested two-phasic tarsal liquid of insects having soft attachment pads [hairy (Gorb et al. 2001) or not (Attygalle et al. 2000; Federle et al. 2002; Vötsch et al. 2002)] might also be found in some beetle species (Betz 2003). Yet, in no case has the hydrophilic liquid been successfully characterised. For most beetles, the liquid recovered from footprints had been postulated to comprise the same set of lipophilic substances as the cuticular lipids (Attygalle et al. 2000; Ishii 1987; Kosaki and Yamaoka 1996), but only recently has the chemical congruence been evidenced by means of direct sampling [solid phase microextraction (SPME)] of tarsi and elytra of the Colorado potato beetle, *Leptinotarsa decemlineata*, and the green dock leaf beetle, *Gastrophysa viridula* (SF Geiselhardt et al. 2009, 2010). In *L. decemlineata* it also has been evidenced that not only the tarsal lipids which are secreted by specialised tarsal glands, but also the cuticular lipids are at least partially liquid, and that both pools are involved in constant substance exchange (SF Geiselhardt et al. 2010).

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With the present study, we aim to show that the chemical congruence of cuticular and tarsal lipids is a general principle in beetles. For this, we compare SPME samples of tarsi and elytra of a representative selection of beetle species with tarsal attachment structures by means of a cluster analysis based on the quantitative and qualitative similarity of the substance compositions. We also discuss possible consequences of a connection between tarsal and cuticular lipids.

Materials and methods

Beetles

Most beetles were collected in and around Freiburg (Germany) during the field seasons of 2005–2007 (see Online Resource 1). Additional species were collected in Bietigheim/Baden, Achern, Bayreuth (all Germany), at the Kaiserstuhl (Germany), or in Illmitz (Austria). *Phaedon cochleariae* was obtained from laboratory breeding at the Freie Universität Berlin (Department of Angewandte Zoologie, Germany) and *Nicrophorus nepalensis* from laboratory breeding at the Universität Freiburg (Department of Biologie I). All of these species possess tarsi that are adapted to life on smooth surfaces, i.e. that are widened to some extent and bear pads of adhesive hairs (Stork 1980; own observations).

Chemical investigations

Solid phase microextraction

The cuticular lipid profile of the elytra was obtained by gently rubbing a 100 µm PDMS fibre (polydimethylsiloxane; Supelco, Bellefonte, PA, USA; colour code red) over the elytra of a beetle which was held in forceps, for 4 min.

The tarsal chemicals were sampled by rubbing the tarsi of an immobilised beetle over a 100 µm PDMS fibre. Details for both methods are given in SF Geiselhardt et al. (2009).

Chemical analysis

Samples were run on a HP 6890 gas chromatograph (GC) with a split/splitless injector (250°C, manual injection). A fused silica column (DB-1, 30 m × 0.25 mm ID, 0.25 µm, J&W Scientific, Folsom, CA) was used with a helium flow of 1 ml/min. The temperature was programmed from 100°C to 300°C at a rate of 6°C/min; 20 min hold at 300°C. The FID was run with 40 ml H_2/min and 450 ml air/min at 300°C.

Chemical identifications were performed on a coupled gas chromatography–mass spectrometer system (HP 6890 series GC–HP 5973 MSD), and electron impact ionisation was 70 eV. GC conditions were as described above. *n*-alkanes, methyl-branched alkanes and *n*-alkenes were identified by their mass spectra (Akino et al. 2002; Haverty et al. 1996; Nelson and Sukkestad 1970; Nelson et al. 1972; Pomonis 1989; Pomonis et al. 1980) and corroborated by their retention indices (Carlson et al. 1998; Pomonis et al. 1989; Schulz 2001). The location of double bonds was determined by means of DMDS derivatisation and confirmed by comparison of mass spectra and retention indices with those of synthetic standards. Hexanoates of secondary alcohols in *Lagria hirta* were characterised by an intense base peak at *m*/*z* 99 in combination with a peak at *m*/*z* 117. Positions of the hydroxyl group were determined as described for acetates by Holotík et al. (1976).

All peaks in the chromatograms were manually integrated and the relative proportions of the peak areas were calculated. Medians of all sampled individuals were determined separately for SPME samples of tarsi and elytra and subjected to a cluster analysis (see below). The number of samples used per species is given in Fig. 1.

Fig. 1 Dendrogram constructed from the cluster analysis of SPME samples of tarsi (T) and elytra (E) of the species investigated with UPGMA linkage. Calculation of distances is based on the product of Nei's distances and Jaccard's distances (I × J). Abbreviations of families: Attelabidae (Atte), Cerambycidae (Cera), Chrysomelidae (Chry), Coccinelldiae (Cocc), Curculionidae (Curc), Oedemeridae (Oede), Silphidae (Silp), Tenebrionidae (Tene). *Numbers in brackets* are numbers of individuals sampled per branch ▶

Cluster analysis

For quantification of the similarities of different cuticular lipid patterns, we calculated the Nei's indices (*I*) for all species pairs (Gush et al. 1985; Kaib et al. 1991; Nowbahari et al. 1990):

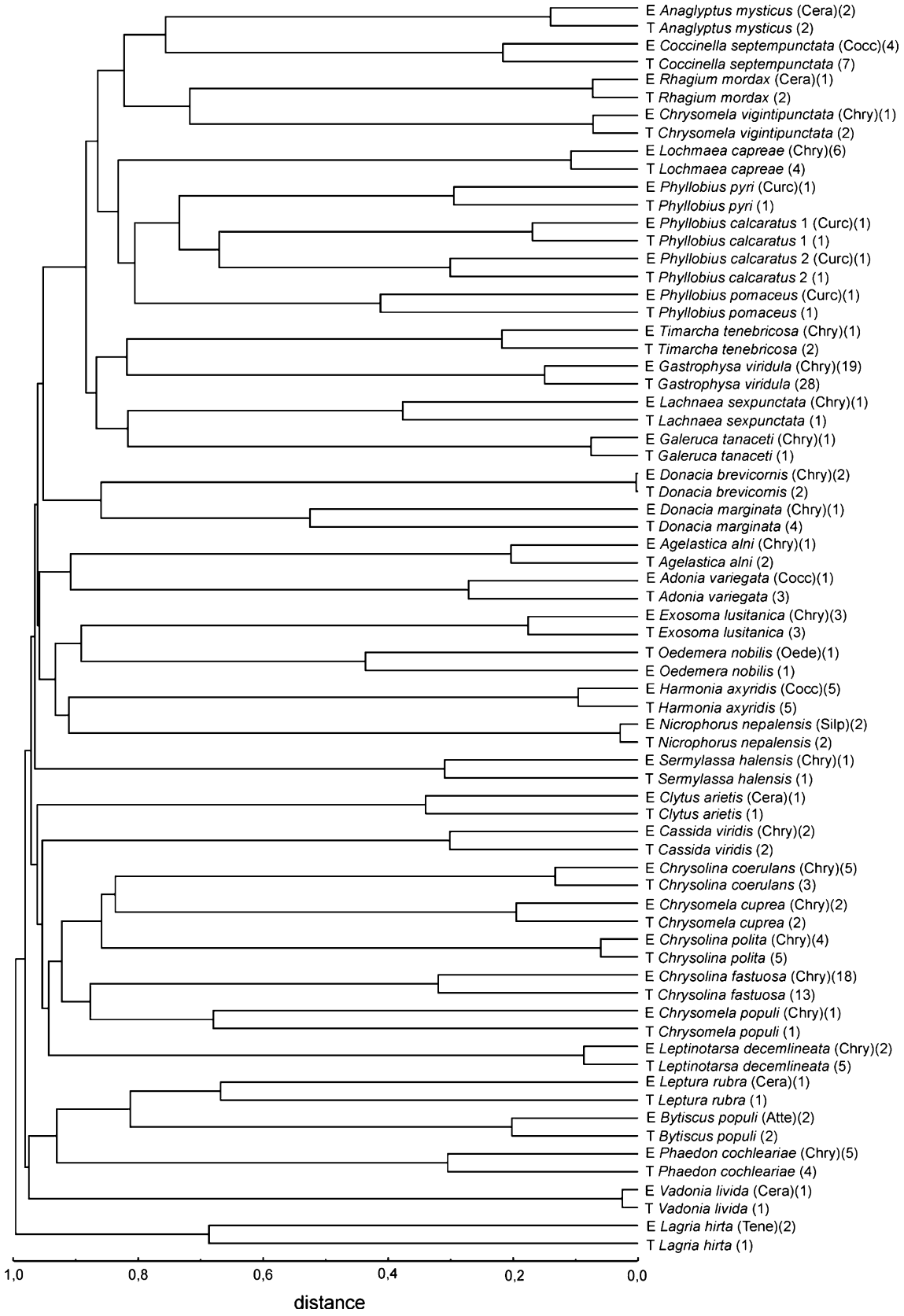
$$I = \frac{\sum x_i y_i}{\sqrt{\sum x_i^2 \sum y_i^2}}$$

where x and y are the mean relative proportions of component i in two species, respectively. However, since Nei's indices are based on the quantity of individual components, small peaks carry little weight. To compensate this, we additionally calculated Jaccard's indices (*J*; Hamers et al. 1989; Jaccard 1901), which rate whether or not a substance is present:

$$J = \frac{C_{AB}}{A + B - C_{AB}}$$

Here, A and B are the number of components of two species, respectively, and C_{AB} is the number of components, the two species share.

For a combined evaluation, we multiplied I and J and used the joint distance ($D = 1 - I \times J$) for the cluster


E Anaglyptus mysticus (Cera)(2)
T Anaglyptus mysticus (2)
E Coccinella septempunctata (Cocc)(4)
T Coccinella septempunctata (7)
E Rhagium mordax (Cera)(1)
T Rhagium mordax (2)
E Chrysomela vigintipunctata (Chry)(1)
T Chrysomela vigintipunctata (2)
E Lochmaea capreae (Chry)(6)
T Lochmaea capreae (4)
E Phyllobius pyri (Curc)(1)
T Phyllobius pyri (1)
E Phyllobius calcaratus 1 (Curc)(1)
T Phyllobius calcaratus 1 (1)
E Phyllobius calcaratus 2 (Curc)(1)
T Phyllobius calcaratus 2 (1)
E Phyllobius pomaceus (Curc)(1)
T Phyllobius pomaceus (1)
E Timarcha tenebricosa (Chry)(1)
T Timarcha tenebricosa (2)
E Gastrophysa viridula (Chry)(19)
T Gastrophysa viridula (28)
E Lachnaea sexpunctata (Chry)(1)
T Lachnaea sexpunctata (1)
E Galeruca tanaceti (Chry)(1)
T Galeruca tanaceti (1)
E Donacia brevicornis (Chry)(2)
T Donacia brevicornis (2)
E Donacia marginata (Chry)(1)
T Donacia marginata (4)
E Agelastica alni (Chry)(1)
T Agelastica alni (2)
E Adonia variegata (Cocc)(1)
T Adonia variegata (3)
E Exosoma lusitanica (Chry)(3)
T Exosoma lusitanica (3)
T Oedemera nobilis (Oede)(1)
E Oedemera nobilis (1)
E Harmonia axyridis (Cocc)(5)
T Harmonia axyridis (5)
E Nicrophorus nepalensis (Silp)(2)
T Nicrophorus nepalensis (2)
E Sermylassa halensis (Chry)(1)
T Sermylassa halensis (1)
E Clytus arietis (Cera)(1)
T Clytus arietis (1)
E Cassida viridis (Chry)(2)
T Cassida viridis (2)
E Chrysolina coerulans (Chry)(5)
T Chrysolina coerulans (3)
E Chrysomela cuprea (Chry)(2)
T Chrysomela cuprea (2)
E Chrysolina polita (Chry)(4)
T Chrysolina polita (5)
E Chrysolina fastuosa (Chry)(18)
T Chrysolina fastuosa (13)
E Chrysomela populi (Chry)(1)
T Chrysomela populi (1)
E Leptinotarsa decemlineata (Chry)(2)
T Leptinotarsa decemlineata (5)
E Leptura rubra (Cera)(1)
T Leptura rubra (1)
E Bytiscus populi (Atte)(2)
T Bytiscus populi (2)
E Phaedon cochleariae (Chry)(5)
T Phaedon cochleariae (4)
E Vadonia livida (Cera)(1)
T Vadonia livida (1)
E Lagria hirta (Tene)(2)
T Lagria hirta (1)
1,0
0,8
0,6
0,4
0,2
0,0
distance

analysis with unweighted pair-group average method (UPGMA) for linkage.

Results and discussion

Investigating 35 plant-inhabiting beetle species out of 8 families, the present study provides a broad survey of the chemical composition of tarsal and cuticular chemicals in beetles sampled by means of contact SPME. A total of 421 different cuticular components were detected with carbon backbones ranging from C_{20} to C_{39}. Individual samples contained between 9 (*Leptura rubra* tarsi) and 96 components (*Chrysomela virgintipunctata* elytra). The components are characteristic for insect cuticular lipids (e.g. Blomquist and Bagnères 2010; Howard and Blomquist 2005; Lockey 1985 and literature therein), comprising *n*-alkanes, mono-, di-, tri- and tetramethyl-branched alkanes (Blomquist 2010), unsaturated hydrocarbons with 1–5 double-bonds (e.g. Page et al. 2002; Steiger et al. 2007), methyl-branched olefins (e.g. Blomquist 2010; S Geiselhardt et al. 2009; SF Geiselhardt et al. 2009) and in a few cases non-hydrocarbon components, such as esters, aldehydes, ketones or alcohols (Buckner 2010; for details on the composition see Online Resource 1). *n*-alkanes (35 species), monomethylalkanes (34 species), and dimethylalkanes (33 species) were nearly omnipresent in our survey, followed by alkenes (27 species) and trimethylalkanes (20 species). Tetramethylalkanes (8 species), dienes (7 species), methylalkenes (3 species), trienes (2 species), tetraenes, pentaenes and methylalkadienes (1 species each) were less abundant. The frequencies of the occurrence of these substance classes largely correspond to the results observed for ants (Martin and Drijfhout 2009) and may reflect a general pattern in insects.

To evaluate the chemical similarity of the cuticular lipids and tarsal secretion of the investigated beetles, we performed a cluster analysis based on the product $I \times J$ of Nei's index (*I*; Gush et al. 1985; Kaib et al. 1991; Nowbahari et al. 1990) and Jaccard's index (*J*; Hamers et al. 1989; Jaccard 1901). This method includes both, differences in qualitative substance composition and relative abundance of substances. Each species formed a cluster consisting of SPME samples of tarsi and elytra, respectively (Fig. 1). The chemical similarity between the two is supported by a great distance to the next knot, which indicates the difference of the chemical profiles of adjacent species in the cluster. The recovered cluster, therefore, indicates a high similarity of the chemical composition of tarsal and cuticular lipids in the investigated beetle species.

The dendrogram was rooted by *L. hirta* (Tenebrionidae) that, based on an extremely low proportion of hydrocarbons in the cuticular lipids (4% on elytra), showed the greatest chemical distances to all other species. The cuticular and tarsal lipids of *L. hirta* are characterised by a series of hexanoates of secondary alcohols (C_{21}–C_{25}) that have not been previously reported for insects.

With the exception of the members of the genera *Phyllobius* (Curculionidae) and *Donacia*, which are grouped together, the distribution of the species within the whole cluster appears to be random, or at least not correlated with taxonomical relationships. On a higher taxonomic level, no grouping was recovered whatsoever, so that conclusions about beetle chemotaxonomy—if possible at all—cannot be drawn from this kind of data set. These findings correspond to a comparative study of ant cuticular hydrocarbons where no correlation between cuticular hydrocarbon similarity and phylogeny was recovered as well (Martin and Drijfhout 2009).

A chemical congruence of tarsal and cuticular chemicals in beetles already had been postulated based on footprint sampling of a leaf beetle (Attygalle et al. 2000) and a few ladybird species (Ishii 1987; Kosaki and Yamaoka 1996). Recently, this has been confirmed for the leaf beetles *L. decemlineata* and *G. viridula* (Chrysomelidae) by means of direct sampling using the method applied in the present study (SF Geiselhardt et al. 2009, 2010). All those investigations, however, have dealt with individual species.

In the present study, we have chosen a representative selection of mostly plant-inhabiting beetles, in which all possess widened tarsal segments and adhesive hairs (Stork 1980; own observations), presumably connected to specialised glandular cells (Betz 2003; SF Geiselhardt et al. 2010). Our focus is on the Chrysomeloidea and Curculionoidea (29 species), where broadened tarsi and adhesive devices seem to be a ubiquitous character that may have evolved in adaptation to life on smooth plant surfaces. In addition, three representatives of the Coccinellidae, which also mostly possess widened tarsi were chosen, and one representative of the Oedemeridae, Tenebrionidae and Silphidae, respectively, where widened tarsi are rather exceptional.

The data presented here demonstrate that the chemical profiles of tarsi and elytra are identical in all investigated species. Our cluster analysis, therefore, shows that the previously introduced congruence of tarsal and cuticular lipids holds true not only for individual beetle species, but for a taxonomically broad selection of species that possess widened tarsi and specialised attachment structures.

In beetle species without morphological adaptations to tarsal attachment, the tarsal surface can be seen simply as a part of the body surface in general. A chemical congruence of tarsal and cuticular lipids, therefore, would be expected in these beetle species. In contrast, the species included in the present study are equipped with specialised attachment structures and at least some possess glands, which seem to

be involved in the secretion of the adhesive liquid (SF Geiselhardt et al. 2010). Hence, the locally restricted production of a specialised formula with optimised adhesive properties would be physiologically possible.

Yet, not only the tarsal secretion is liquid, but also the film of surface lipids on the cuticle, and in some butterfly species, topographical differences in the cuticular lipid composition have been observed (Arsene et al. 2002; Böröczky et al. 2008). In *L. decemlineata*, however, preceding experiments have shown that the substance pools of the elytral cuticle and tarsi are in constant exchange of material (SF Geiselhardt et al. 2010). Since the cuticular lipid composition of all species investigated in the present study and a large number of additional species (Geiselhardt et al. in preparation) are very similar, we suggest the substance exchange to generally result in a homogeneously composed liquid film on tarsi and cuticula, as we found it in *L. decemlineata*.

The present study evidences the similarity of tarsal and cuticular chemicals of a large number of species with convergently developed widened tarsi (Stork 1980). While the role of non-lipophilic components in the context of tarsal attachment remains to be investigated, our data support the idea that tarsal liquids are part of the cuticular lipid layer (Hasenfuss 1977). Based on our findings, we suggest this to be a widespread principle in beetles. Seeing that the attachment force in liquid-mediated systems seems to depend on the chemical composition of the liquid, future studies, therefore, should consider tarsal attachment and cuticular lipids in the context of each other. Possibly, this is not only true for beetles, but also for representatives of other insect orders with adhesive liquids.

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References

Akino T, Terayama M, Wakamura A, Yamaoka R (2002) Intraspecific variation of cuticular hydrocarbon composition in *Formica japonica* Motschoulsky (Hymenoptera: Formicidae). Zool Sci 19:1155–1165

Arsene C, Schulz S, Van Loon JJA (2002) Chemical polymorphism of the cuticular lipids of the cabbage white *Pieris rapae*. J Chem Ecol 28:2627–2631

Attygalle AB, Aneshansley DJ, Meinwald J, Eisner T (2000) Defense by foot adhesion in a chrysomelid beetle (*Hemisphaerota cyanea*): characterization of the adhesive oil. Zoology 103:1–6

Betz O (2003) Structure of the tarsi in some *Stenus* species (Coleoptera, Staphylinidae): external morphology, ultrastructure, and tarsal secretion. J Morph 255:24–43

Betz O, Kölsch G (2004) The role of adhesion in prey capture and predator defence in arthropods. Arthopod Struct Dev 33:3–30

Beutel RG, Gorb SN (2001) Ultrastructure of attachment specializations of hexapods (Arthropoda): evolutionary patterns inferred from a revised ordinal phylogeny. J Zool Syst Evol Res 39:177–207

Beutel RG, Gorb SN (2006) A revised interpretation of the evolution of attachment structures in hexapoda with special emphasis on Mantophasmatodea. Arthropod Syst Phyl 64:3–25

Blomquist GJ (2010) Structure and analysis of insect hydrocarbons. In: Blomquist GJ, Bagnères AG (eds) Insect hydrocarbons. Cambridge University Press, Cambridge

Blomquist GJ, Bagnères AG (eds) (2010) Insect hydrocarbons: biology, biochemistry and chemical ecology. Cambridge University Press, Cambridge

Böröczky K, Park KC, Minard RD, Jones TH, Baker TC, Tumlinson JH (2008) Differences in cuticular lipid composition of the antennae of *Helicoverpa zea*, *Heliothis virescens*, and *Manduca sexta*. J Insect Physiol 54:1385–1391

Buckner JS (2010) Oxygenated derivatives of hydrocarbons. In: Blomquist GJ, Bagnères AG (eds) Insect hydrocarbons. Cambridge University Press, Cambridge

Carlson DA, Bernier UR, Sutton BD (1998) Elution patterns from capillary GC for methyl-branched alkanes. J Chem Ecol 24:1845–1865

Drechsler P, Federle W (2006) Biomechanics of smooth adhesive pads in insects: influence of tarsal secretion on attachment performance. J Comp Physiol A 192:1213–1222

Federle W, Riehle M, Curtis ASG, Full RJ (2002) An integrative study of insect adhesion: mechanics and wet adhesion of pretarsal pads in ants. Integr Comp Biol 42:1100–1106

Geiselhardt S, Otte T, Hilker M (2009) The role of cuticular hydrocarbons in male mating behavior of the mustard leaf beetle, *Phaedon cochleariae* (F.). J Chem Ecol 35:1162–1171

Geiselhardt SF, Geiselhardt S, Peschke K (2009) Comparison of tarsal and cuticular chemistry in the leaf beetle *Gastrophysa viridula* (Coleoptera: Chrysomelidae) and an evaluation of solid-phase microextraction and solvent extraction techniques. Chemoecology 19:185–193

Geiselhardt SF, Lamm S, Gack C, Peschke K (2010) Interaction of liquid epicuticular hydrocarbons and tarsal adhesive secretion in *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). J Comp Physiol A 196:369–378

Gorb S, Gorb E, Kastner V (2001) Scale effects on the attachment pads and friction forces in syrphid flies (Diptera, Syrphidae). J Exp Biol 204:1421–1431

Gush TJ, Bentley BL, Prestwich GD, Thorne BL (1985) Chemical variation in defensive secretions of four species of *Nasutitermes*. Biochem Syst Ecol 13:329–336

Hamers L, Hemeryck Y, Herweyers G, Janssen M, Keters H, Rousseau R, Vanhoutte A (1989) Similarity measures in scientometric research—the jaccard index versus salton cosine formula. Inf Process Manag 25:315–318

Hasenfuss I (1977) Die Herkunft der Adhäsionsflüssigkeit bei Insekten. Zoomorphology 87:51–64

Haverty MI, Grace JK, Nelson LJ, Yamamoto RT (1996) Intercaste, intercolony, and temporal variation in cuticular hydrocarbons of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). J Chem Ecol 22:1813–1834

Holotík Š, Leško J, Krupčík J, Tesařik K (1976) Identification of acetates of secondary straight-chain alcohols by gas chromatography–mass spectrometry. Chromatographia 9:443–446

Howard RW, Blomquist GJ (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu Rev Entomol 50:371–393

Ishii S (1987) Adhesion of a leaf feeding ladybird *Epilachna vigintioctomaculta* (Coleoptera: Coccinellidae) on a vertically smooth surface. Appl Entomol Zool 22:222–228

Jaccard P (1901) Etude comparative de la distribution florale dans une portion des Alpes et des Jura. Bull Soc Vaudoise Sci Nat 37:547–579

Kaib M, Brandl R, Bagine RKN (1991) Cuticular hydrocarbon profiles: a valuable tool in termite taxonomy. Naturwissenschaften 78:176–179

Kosaki A, Yamaoka R (1996) Chemical composition of footprints and cuticula lipids of three species of lady beetles. Jpn J Appl Entomol Zool 40:47–53

Lockey KH (1985) Insect cuticular lipids. Comp Biochem Physiol B 81:263–273

Martin S, Drijfhout F (2009) A review of ant cuticular hydrocarbons. J Chem Ecol 35:1151–1161

McFarlane JS, Tabor D (1950) Adhesion of solids and the effect of surface films. Proc R Soc Lond A 202:224–243

Nelson DR, Sukkestad DR (1970) Normal and branched aliphatic hydrocarbons from eggs of the tobacco hornworm. Biochemistry 9:4601–4611

Nelson DR, Sukkestad DR, Zaylskie RG (1972) Mass spectra of methyl-branched hydrocarbons from eggs of the tobacco hornworm. J Lipid Res 13:413–421

Nowbahari E, Lenoir A, Clément JL, Lange C, Bagnères AG, Joulie C (1990) Individual, geographical and experimental variation of cuticular hydrocarbons of the ant *Cataglyphis cursor* (Hymenoptera: Formicidae): their use in nest and subspecies recognition. Biochem Syst Ecol 18:63–73

Page M, Nelson LJ, Forschler BT, Haverty MI (2002) Cuticular hydrocarbons suggest three lineages in *Reticulitermes* (Isoptera: Rhinotermitidae) from North America. Comp Biochem Physiol B 131:305–324

Pomonis JG (1989) Cuticular hydrocarbons of the screwworm, *Cochliomyia hominivorax* (Diptera: Calliphoridae). Isolation, identification, and quantification as a function of age, sex and irradiation. J Chem Ecol 15:2301–2317

Pomonis JG, Nelson DR, Fatland CL (1980) Insect hydrocarbons. 2. Mass spectra of dimethylalkanes and the effect of the number of methylene units between groups on fragmentation. J Chem Ecol 6:965–972

Pomonis JG, Hakk H, Fatland C (1989) Synthetic methyl- and dimethylalkanes. J Chem Ecol 15:2319–2332

Schulz S (2001) Composition of the silk lipids of the spider *Nephila clavipes*. Lipids 36:637–647

Steiger S, Peschke K, Francke W, Müller JK (2007) The smell of parents: breeding status influences cuticular hydrocarbon pattern in the burying beetle *Nicrophorus vespilloides*. Proc R Soc Lond B 274:2211–2220

Stork NE (1980) A scanning electron microscope study of tarsal adhesive setae in the Coleoptera. Zool J Linn Soc 68:173–306

Vötsch W, Nicholson G, Müller R, Stierhof Y-D, Gorb S, Schwarz U (2002) Chemical composition of the attachment pad secretion of the locust *Locusta migratoria*. Insect Biochem Mol Biol 32:1605–1613