

Composition of cuticular lipids in the pteromalid wasp *Lariophagus distinguendus* is host dependent

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Abstract

The insect cuticle is covered by a thin layer of hydrocarbons not only preventing desiccation but also playing an important role in the sexual communication of several species. In the pteromalid wasp *Lariophagus distinguendus*, a parasitoid of grain infesting beetles, female cuticular hydrocarbons (CHCs) elicit male courtship behaviour. We analyzed the CHC profiles of male and female *L. distinguendus* wasps reared on different beetle hosts by coupled gas chromatography- mass spectrometry (GC-MS). Statistical analysis of the data revealed significant differences between strains reared on different hosts, while spatially isolated strains reared on the same host produced similar profiles. CHC profiles of parasitoids reared on *Stegobium paniceum* were statistically distinguishable from those of wasps reared on all other hosts. A host shift from *Sitophilus granarius* to *S. paniceum* resulted in distinguishable CHC profiles of *L. distinguendus* females after only one generation. Considering the role of CHCs as contact sex pheromones, our data suggest that host shifts in parasitic wasps might lead to reproductive isolation of host races due to the modification of the cuticular semiochemistry.

Keywords: contact sex pheromone, cuticular hydrocarbons (CHCs), host shift, parasitic wasp

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Introduction

Cuticular hydrocarbons (CHCs) of insects function mainly as a water barrier to avoid desiccation, but also play an important role in intraspecific communication. Because of their low volatility, they act mostly over short distances as contact pheromones (Singer, 1998; Gibbs, 2002; Blomquist & Bagnères, 2010). CHC profiles are complex mixtures of aliphatic long-chain alkanes and alkenes, as well as methyl-branched alkanes. Described functions of CHCs comprise the mediation of recognition, aggregation, dispersal, alarm and

sexual behaviour in insects (Howard, 1993; Tillmann *et al.*, 1999; Blomquist & Bagnères, 2010). While social insects also use CHCs for recognition and interaction with nestmates and as fertility and dominance signals (Singer, 1998; Liebig, 2010), solitary insects mainly use CHCs for the discrimination of conspecifics and enemies, location of mating partners and the elicitation of courtship behaviour (Ruther *et al.*, 2011). Evidence for solitary insects using CHCs as contact sex pheromones comes from several insect orders, for example the Coleoptera (Buprestidae: Lelito *et al.*, 2009; Silk *et al.*, 2009; Cerambycidae: Ginzel, 2010; Chrysomelidae: Sugeno *et al.*, 2006; Peterson *et al.*, 2007; Geiselhardt *et al.*, 2009), Diptera (Drosophilidae, Glossinidae and Muscidae: Wicker-Thomas, 2007; Ferveur & Cobb, 2010) and Hymenoptera (Syvertsen *et al.*, 1995; Schiestl *et al.*, 2000; Sullivan, 2002; Mant *et al.*, 2005; Steiner *et al.*, 2005, 2006, 2007). Within the parasitic wasp

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Table 1. Investigated *Lariophagus distinguendus* strains reared on different beetle hosts.

	Host species	Host substrate	Reared on host since	<i>n</i> ♂	<i>n</i> ♀	Strain-origin
BerSit	<i>Sitophilus granarius</i> (Curculionidae)	wheat-grains	2003	10	12	Berlin
PfoSit	<i>Sitophilus granarius</i> (Curculionidae)	wheat-grains	2005	10	14	Pforzheim
StuSteg	<i>Stegobium paniceum</i> (Anobiidae)	koi fish pellets	2007	10	15	Stuttgart
RavSteg	<i>Stegobium paniceum</i> (Anobiidae)	koi fish pellets	2008	8	14	Ravensburg
BerAca	<i>Acanthoscelides obtectus</i> (Bruchidae)	black-eyed peas	2008	10	15	Berlin
BerLas	<i>Lasioderma serricornis</i> (Anobiidae)	wheat-grains	2004	10	13	Berlin

n, number of samples in chemical analysis.

family Pteromalidae, females of *Roptrocercus xylophagorum* (Sullivan, 2002), *Lariophagus distinguendus* (Steiner *et al.*, 2005, 2007), *Nasonia vitripennis* (Steiner *et al.*, 2006) and *Dibrachys cavus* (Ruther *et al.*, 2011) produce CHCs which act at short-range as contact sex pheromones eliciting courtship behaviour in males.

The diet of an insect can be an important factor influencing its pheromone communication (Landolt & Phillips, 1997; Tillmann *et al.*, 1999; Blomquist, 2010). With respect to CHCs, three ways of acquisition are conceivable which are not mutually exclusive: (a) CHCs may be sequestered from the diet after ingestion, (b) absorbed from the environment, or (c) synthesized *de novo* in oenocytes from dietary precursors (Blomquist & Jackson, 1973; Etges *et al.*, 2006; Bagnères & Blomquist, 2010). In the case of parasitic wasps, the first two ways are of particular interest because, due to their parasitic life cycle, these insects have been suggested to have lost the ability to biosynthesize fatty acids (Visser *et al.*, 2010), i.e. the same machinery involved in CHC biosynthesis (Blomquist, 2010). However, the way how parasitic wasps acquire their CHCs and how the composition is controlled is not well understood. In any case, resources provided by the host should be of crucial importance for the cuticular chemistry.

Because of the influence of diet on the pheromone chemistry of insects, it is reasonable to assume that changes in the diet, e.g. caused by host switches in phytophagous or carnivorous insects, may lead to a breakdown in communication between mating partners and may ultimately contribute to the formation of host races and speciation. In fact, an example for such a host shift-induced breakdown in communication is reported for *Drosophila serrata* and *D. melanogaster*. In these species, the development on different substrates was found to induce differences in the CHC profiles, leading to preferential mating of individuals from the same substrates (Rundle *et al.*, 2005; Sharon *et al.*, 2010). A similar scenario is also thinkable in oligophagous and polyphagous parasitic wasps. Different hosts may provide different pools of precursors for CHC biosynthesis or different CHCs to be sequestered by the wasps. Consequently, feeding on different hosts might lead to differences in the CHC profiles of male and female wasps of one population, causing a breakdown in sexual communication and eventually leading to speciation.

As a first step to study this hypothesis, the present paper examines the influence of hosts on the CHC profile in *Lariophagus distinguendus*, a quasi-gregarious and polyphagous ectoparasitoid of grain infesting beetles (Steidle & Schöller, 1997). Female cuticular hydrocarbons have been shown to arrest males and elicit wing fanning, a typical element of the male courtship behaviour (Steiner *et al.*, 2005). Interestingly, also pupae of both sexes and newly emerged males elicit courtship behaviour in older males. Unlike

females, however, males deactivate the behaviourally active chemicals within 32 h after emergence (Steiner *et al.*, 2005, 2007; Ruther & Steiner, 2008). Thus, CHCs evolved to a sex-specific contact pheromone mediating mate recognition in *L. distinguendus*. We analyzed the CHCs of wasps from six strains reared on four different host species and analyzed the relative composition of the profiles by multivariate statistical methods. Our questions were: Are there differences between the CHC profiles from strains reared on different hosts? Do these differences occur in both sexes? Which compounds account for the differences in CHC profile? Can differences in the CHC profiles be caused by a host shift on an alternative host already within one generation? Do the wasps directly sequester significant amounts of host CHCs? The results are discussed with respect to a possible role of CHCs in prezygotic isolation and sympatric speciation.

Materials and methods

Insects

Six strains of *L. distinguendus* were reared on four different beetle species as hosts as described by Steidle & Schöller (1997) (table 1). The wasp cultures were kept in Petri dishes at 25°C, L16:D08 photoperiod and 50% RH. Freshly emerged wasps were isolated and kept under the same conditions for two days. Afterwards, they were deep frozen and stored at -23°C until they were extracted for chemical analysis.

Chemical analysis

For the analysis of CHCs, three *L. distinguendus* individuals from the same strain and sex were pooled and extracted for 15 min in 30 µl of hexane containing tetracosane (2.6 ng µl⁻¹) as an internal standard. The solvent was evaporated under a gentle stream of nitrogen, and the sample was re-dissolved in 10 µl of hexane. To investigate the possible sequestration of ingested host-derived CHCs by the parasitoid, we also analyzed the CHC profiles of the four beetle hosts both in the larval and in the adult stage following the protocol described above (*n*=3 for each host species and stage, respectively). Aliquots (1 µl in splitless mode) of these extracts were analyzed by coupled gas-chromatography mass spectrometry (GC-MS) on a Shimadzu GCMS-QP2010 Plus quadrupole MS (Shimadzu, Tokyo, Japan) equipped with a 30 m × 0.32 mm I. D. BPX5 forte capillary column (film thickness 0.25 µm) (SGE Analytical Science Europe, Milton Keynes, UK). Helium was used as carrier gas at a constant column flow of 1.73 ml min⁻¹. The oven program started at 150°C and was increased at 3°C min⁻¹ up to 300°C (held for 20 min). The GC effluent was ionized by electron impact ionization at 70 eV; the mass range reached from *m/z* 35 to *m/z* 600.

Relative retention indices (LRI) of methyl-branched and unsaturated hydrocarbons were estimated by co-injection of straight-chain hydrocarbons (van Den Dool & Kratz, 1963). Methyl-branched hydrocarbons were identified by diagnostic ions resulting from the favoured fragmentation at the branching points (Lockey, 1988; Nelson, 1993) and by comparing LRI values with literature data (Carlson *et al.*, 1998; Steiner *et al.*, 2005, 2006, 2007). Positions of the double bonds of unsaturated hydrocarbons were determined by iodine-catalyzed methylthiolation using dimethyl disulphide (Francis & Velant, 1981; Howard, 1993). MS and LRI data of identified compounds were used to build a custom MS library allowing automatic analysis of GC-MS runs with the help of a two-dimensional search algorithm (MS+LRI) using the GC-MS Solution scientific software (Shimadzu) of the mass spectrometer.

Host shift experiment

Female wasps from the BerSit strain kept on *Sitophilus granarius* (F0) were reared for one generation on *Stegobium paniceum* (F1). The CHC profiles of the wasps from the F1 generation were analyzed as described above. The resulting data were compared to those from female wasps, which were reared at the same conditions but without a host shift (F1 *S. granarius*).

Statistical analysis

We integrated the 50 largest peaks (by area) within each run (overlapping compounds were calculated together). All peaks larger than 1% of the whole peak area were selected for further analysis. The absolute amount of each compound was calculated by relating individual peak areas to the internal standard. Statistical analysis was conducted with PAST version 2.01 scientific software (Hammer *et al.*, 2001). We used the non-metric multidimensional scaling (NMDS, Bray-Curtis similarity measure) to visualize the data and the non-parametric MANOVA (NPMANOVA, Bray-Curtis similarity measure of Bonferroni-corrected data) for calculation of the differences between CHC profiles of wasps from the different hosts. Similarity percentage (SIMPER) was used to calculate the individual contribution of each peak to the differences between wasps from different hosts.

Results

The CHC profiles of the wasps consisted mainly of methyl-branched long chain alkanes. For the analysis of female and male profiles, 33 and 30 compounds, respectively, were used (table 2–3). Overall, 83 female and 58 male samples were analyzed. Data of wasps originating from different strains but reared on the same host species (table 1) were pooled for statistical analysis since there were no significant differences in the NPMANOVA analysis between strains reared on the same host-species (*S. granarius* Berlin vs. Pforzheim: $P=1$ (males), $P=0.9285$ (females); *S. paniceum* Stuttgart vs. Ravensburg: $P=1$ (males); permutation $n=10,000$) with the exception of female wasps grown on *Stegobium paniceum* from the Ravensburg and the Pforzheim strain ($P=0.0045$).

Differences in CHC profiles of females reared on different host-species

The NMDS analysis of the CHC profiles of *L. distinguendus* females reared on different beetle species as hosts (fig. 1A) showed a distinct separation between a cluster consisting of wasps from *S. granarius*, *A. obtectus* and *L. serricornis* and a cluster of wasps from *S. paniceum*. While wasps from *S. granarius* and *A. obtectus* overlapped fully, *L. serricornis* was concentrated at one edge of the cluster. This is also reflected in the NPMANOVA analysis, which gave significant differences between all hosts ($P<0.05$; permutation $n=10,000$) with the exception of *S. granarius* and *A. obtectus* ($P=0.1566$). The SIMPER analysis allowed identification of compounds contributing most to the dissimilarity of the CHC profiles of wasps from different beetle hosts. The compounds with the strongest impact were: 3,7,11,15-tetramethyltrtriacontane, 11,21- +11,15-dimethyltrtriacontane, 3,7,11-trimethyltrtriacontane, and an unknown compound with an LRI of 3089 (table 2).

Differences in CHC profiles of males reared on different host-species

In *L. distinguendus* males reared on different beetle species, NMDS indicated also a separation of a cluster consisting of males from *S. granarius* and *A. obtectus* and a cluster consisting of males from *S. paniceum* (fig. 1B). Males from *Lasioderma serricornis* were located in a third cluster intermediate to the others. While wasps from *S. granarius* and *A. obtectus* overlapped, the other clusters did not. This result was also supported by NPMANOVA analysis, which revealed significant differences between all hosts ($P<0.05$; permutation $n=10,000$) with the exception of wasps from *S. granarius* and *A. obtectus* ($P=1$). SIMPER analysis of the male profiles revealed 3,7,11,15-tetramethyltrtriacontane, 13,17-dimethylpentatriacontane, 3-methyltrtriacontane and 11,21- +11,15-dimethyltrtriacontane as compounds with major influence on the dissimilarity of the CHC profiles of wasps from different beetle hosts (table 3).

Host shift experiment

NMDS-analysis of female wasps (fig. 2) showed a clear separation between clusters formed by CHC profiles of wasps reared on *S. granarius* and *S. paniceum* in the F1 generation. This result was supported by NPMANOVA analysis, which revealed significant differences between both strains ($P<0.05$; permutation $n=10,000$). The compounds with the highest influence on the dissimilarity of profiles were 3,7,11,15-tetramethyltrtriacontane, 11,21- +11,15-dimethyltrtriacontane, 13,17-dimethylpentatriacontane and the peak belonging to the co-eluting compounds 15- +13- +11-methyltrtriacontane (SIMPER analysis; table 4).

Comparison of CHC profiles from hosts and parasitoid

To investigate the possible direct sequestration of CHCs from the host into the parasitoid, we compared the CHC profiles of beetle hosts and the respective parasitoids. These analyses revealed that host CHCs cannot account for the observed major differences of the CHC profiles because the CHC profiles of the wasps are generally composed of compounds with higher molecular masses when compared to

Table 2. Similarity Percentage (SIMPER) analysis of the *L. distinguendus* females CHC-profiles (overall average dissimilarity: 29.19).

Compound:	LRI ¹	Contribution	Cumulative%	Mean abund. 1	Mean abund. 2	Mean abund. 3	Mean abund. 4
3,7,11,15-TetraMeC33	3442	3.1	10.46	32.7	50.1	37.1	50.3
11,21- +11,15-DiMeC33	3350	2.053	17.38	22.5	10.2	25.3	24.5
unknown	3089	1.6	22.78	3.43	14	4.46	15.3
3,7,11-TriMeC33	3425	1.517	27.89	16.8	23.4	17.6	22.6
3,7-DiMeC33	3404	1.451	32.79	18.3	20.9	20.9	22.7
15- +13- +11-MeC33	3328	1.433	37.62	17.9	10.6	19	20.2
4,8-DiMeC34	3477	1.277	41.93	6.23	10.5	9.22	8.88
3-MeC33	3374	1.256	46.17	15.5	22.7	18.8	21.8
13,17-DiMeC35	3541	1.256	50.4	21.8	19.4	21.5	22.3
3-MeC27	2773	1.143	54.26	5.35	12.8	3.65	8.61
C33:1(9)	3280	1.106	57.99	5.38	13	5.11	3.45
unknown	3394	0.975	61.28	2.92	5.61	2.65	3.13
3-MeC35 + 5,9DiMeC35	3569	0.9647	64.53	2.85	10.3	3.47	5.03
C27:1(9) + 3-MeC26	2675	0.9599	67.77	0.467	8.46	0	0
3,7-DiMeC27	2805	0.7861	70.42	3.25	7.02	0.911	0.626
unknown	3562	0.775	73.03	3.82	0.696	4.65	4.85
unknown	3411	0.7719	75.64	0.928	4.2	1.68	2.26
17- +15- +13- +11-MeC35	3517	0.7222	78.07	7.87	12.4	8.24	11.5
C33	3300	0.7127	80.48	5.29	1.52	7.55	6.33
3,7,11,15-TetraMeC35	3631	0.6681	82.73	4.94	5.57	6.45	10.4
C25	2500	0.6353	84.87	3.71	5.17	0.655	1.17
Cholesterol	3122	0.4739	86.47	4.67	4.77	3.56	3.04
C27	2700	0.4674	88.05	4.05	4.17	2.11	1.45
15- +13- +11-MeC31	3130	0.4286	89.49	4.06	1.89	2.83	5.79
C29:1(9)	2877	0.4049	90.86	0.283	3.6	0	0
unknown	3606	0.4026	92.22	0.653	3.47	0.739	0.715
C30:1(9)	3017	0.3987	93.56	1.59	1.58	2.13	5.84
C33	3300	0.3958	94.9	1.03	0.376	1.1	4.03
5,9-DiMeC25	2577	0.3336	96.02	0.283	2.81	0	0.138
19- +17- +15- +13- +11-MeC37	3711	0.3084	97.06	4	3.92	4.05	4.75
C32 + 3,7-DiMeC31	3204	0.2523	97.91	2.68	3.68	2.44	2.84
3-MeC31	3173	0.2286	98.68	1.87	3.16	1.7	2.59
3,7,11-TriMeC31	3425	0.2191	99.42	2.95	3.42	2.66	3.71

¹ Linear Retention Index according to van Den Dool & Kratz (1963).

larval and adult stages of the respective beetle hosts (for comparative fingerprint chromatograms see figs S1–4 in the supplementary material). The major compound of the parasitoids are almost absent in the hosts. Conversely, several major compounds of the beetle hosts occurred only in traces in the CHC profiles of the wasps or were completely absent. Apart from *A. obtectus*, CHCs were hardly present in cuticular extracts from larvae.

Discussion

The chemical analysis of CHC profiles of female and male *L. distinguendus* wasps reared on different beetle hosts revealed significant quantitative differences. These were not only observed between strains from different hosts but also between individuals from the same strain which were reared on the two hosts, *S. granarius* and *S. paniceum*. In contrast, the profiles of wasp strains reared on the same host species were similar with the exception of female wasps from the Ravensburg and Pforzheim strain reared on *S. paniceum*. Furthermore, some of the compounds with major influence on the differences between wasp strains reared on *S. granarius* or *S. paniceum* were also found to be important in the host-shift experiment. Thus, the CHC profiles of *L. distinguendus* are indeed host dependent.

Remarkably, the differences in CHC profiles between wasps from *S. granarius* and *S. paniceum* were present already after one generation and did not require several generations to develop. This indicates that the presence and quantity of compounds in the CHC profiles do not depend on strain-related features but are presumably caused by host-dependent precursors in the diet of the wasps. Although direct incorporation of host CHCs can only be demonstrated by labelling experiments (Blomquist & Jackson, 1973), which, to our knowledge, have never been performed in parasitic wasps, this is unlikely in *L. distinguendus*. Neither larvae nor adults of the four beetle hosts had significant amounts of the parasitoids' major CHCs on their cuticle, and vice versa many major components of the beetle CHCs were absent from the wasps' cuticle or occurred only in traces. This suggests that the host species influences the wasps' own CHC metabolism rather than serving as a direct source for CHC sequestration. The published literature on parasitoid/host CHCs does not provide a clear picture of whether parasitoids are able to sequester significant amounts of host CHCs or not. Some species share major components with their hosts (see for instance Howard & Liang, 1993; Howard & Infante, 1996); whereas, in other studies, host and parasitoid profiles differed clearly (Howard & Perez-Lachaud, 2002). Like in the present study, the qualitative composition of the CHC profile was largely independent from the host in the bethylid wasp

Table 3. Similarity Percentage (SIMPER) analysis of the *L. distinguendus* males CHC-profiles (Overall average dissimilarity: 30.92).

Compound:	LRI ¹	Contribution	Cumulative%	Mean abund. 1	Mean abund. 2	Mean abund. 3	Mean abund. 4
3,7,11,15-TetraMeC33	3442	3.927	12.7	10.5	24.2	9.22	21.2
13,17-DiMeC35	3541	3.04	22.53	15.5	25.1	12.9	16.2
3-MeC33	3376	2.535	30.73	8.97	19	8.36	12.7
11,21- +11,15-DiMeC33	3351	2.474	38.73	19.9	13.1	19.2	15.8
3,7,11-TriMeC33	3426	1.916	44.93	5.58	12.5	5.72	8.6
unknown	3567	1.854	50.92	2.25	10.4	2.41	3.73
15- +13- +11-MeC33	3330	1.589	56.06	12.2	7.8	8.55	7.02
3,7-DiMeC33	3406	1.462	60.79	8.98	12.3	8.3	11.6
C33	3300	1.294	64.97	6.36	2.19	6.21	7.27
17- +15- +13- +11-MeC35	3520	1.227	68.94	4.26	8.56	3.25	4.65
unknown	3396	0.8648	71.74	0.47	4.29	0.452	0.569
unknown	3126	0.8101	74.36	3.38	0.912	2.51	1.25
C25	2500	0.734	76.73	2.85	1.82	0.986	0.0863
3,7,11,15-TetraMeC35	3632	0.6825	78.94	2.13	3.22	1.6	5
unknown	3816	0.6115	80.92	1.69	0.0115	1.24	2.78
15- +13- +11-MeC31	3132	0.6087	82.88	2.88	0.401	1.11	0.813
19- +17- +15- +13- +11-MeC37	3713	0.597	84.81	4.29	4.81	3.47	4.95
C33:1(9)	3282	0.5588	86.62	2.33	1.11	2.02	0.496
C27	2700	0.4812	88.18	2.01	0.869	0.973	0.164
C32+3,7-DiMeC31	3206	0.4105	89.51	0.74	1.91	0.354	0.0949
C31	3100	0.4002	90.8	2.15	0.752	2.06	1.65
unknown	3453	0.3946	92.08	0.201	0.4	0.323	2.53
4,8-DiMeC34	3478	0.3849	93.32	2.06	2.82	2.22	2.37
unknown	3589	0.3541	94.47	0.0913	1.72	0.153	0.331
3-MeC31	3175	0.3456	95.58	1.15	2.27	0.992	0.7
unknown	3150	0.3332	96.66	1.63	0.536	1.47	0.557
unknown	2797	0.3173	97.69	1.23	0.643	1.78	0.889
C29	2900	0.2743	98.57	1.41	0.476	1.16	0.627
3247	3249	0.2208	99.29	1.33	1.07	0.822	0.703
unknown	3340	0.2204	100	1.11	0.667	1.42	0.98

¹ Linear Retention Index according to van Den Dool & Kratz (1963).

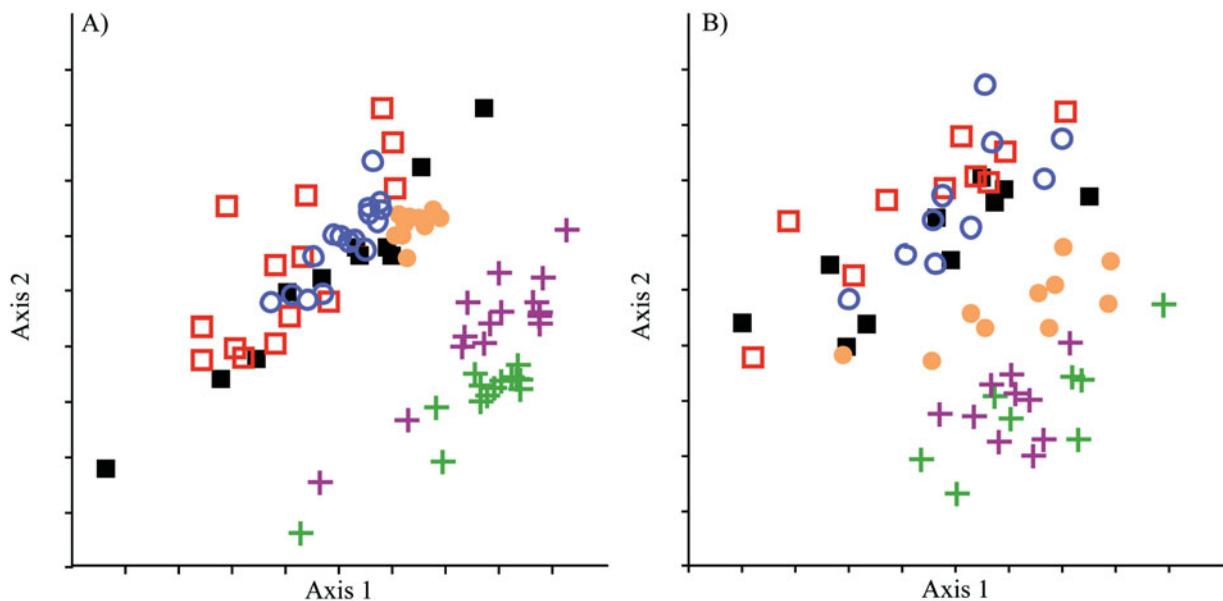


Fig. 1. Non-metric multidimensional scaling (NMDS; Bray-Curtis similarity measure) of the CHC profiles of *L. distinguendus* females (A) and males (B). Hosts: □ = *S. granarius* (Pforzheim); ■, *S. granarius* (Berlin); +, *S. paniceum* (Ravensburg); ○, *S. paniceum* (Stuttgart); o, *A. obtectus* (Berlin); ●, *L. serricornis* (Berlin).

Table 4. Similarity Percentage (SIMPER) analysis of CHC-profiles of *L. distinguendus* wasps from the host shift experiment (Overall average dissimilarity: 25.76).

Compound:	LRI ¹	Contribution	Cumulative%	Mean abund. 1	Mean abund. 2
3,7,11,15-TetraMeC33	3443	4.507	17.49	62.1	39.9
11,21- +11,15-DiMeC33	3350	3.206	29.94	38.6	24
13,17-DiMeC35	3542	2.185	38.42	30.9	21.1
15- +13- +11-MeC33	3329	1.909	45.83	25.8	18.7
3-MeC33	3374	1.433	51.39	24.2	17.9
3,7,11-TriMeC33	3425	1.412	56.87	24.5	19.1
unknown	3089	1.294	61.89	10.7	4.16
3,7-DiMeC33	3405	0.9706	65.66	21.8	20.4
unknown	3124	0.9154	69.21	0.142	5.26
C30:1(9)	3016	0.8262	72.42	5.74	1.37
C33:1(9)	3280	0.8067	75.55	1.54	5.71
4,8-DiMeC32	3285	0.6666	78.14	3.85	0.712
3-MeC27	2773	0.6603	80.7	5.99	4.48
17- +15- +13- +11-MeC35	3517	0.5934	83	9.96	8.16
unknown	2173	0.5399	85.1	0	3.31
4,8-DiMeC34	3477	0.5168	87.1	2.55	5.27
C33	3300	0.51	89.08	3.63	6.19
15- +13- +11-MeC31	3129	0.4221	90.72	4.88	4.06
3,7,11,15-TetraMeC35	3631	0.3923	92.24	5.66	6.26
C25	2500	0.3136	93.46	0.0577	1.83
unknown	2801	0.2619	94.48	0.778	1.88
unknown	3394	0.2507	95.45	1.39	1.9
C27	2700	0.2479	96.41	1.05	2.14
3,7,11-TriMeC31	3227	0.2389	97.34	2.64	2.91
19- +17- +15- +13- +11-MeC37	3712	0.229	98.23	3.74	3.28
C32+3,7-DiMeC31	3204	0.1951	98.99	2.26	2.68
3-MeC31	3173	0.1365	99.52	1.84	1.93
C29	2900	0.1247	100	1.35	1.78

¹ Linear Retention Index according to van Den Dool & Kratz (1963).

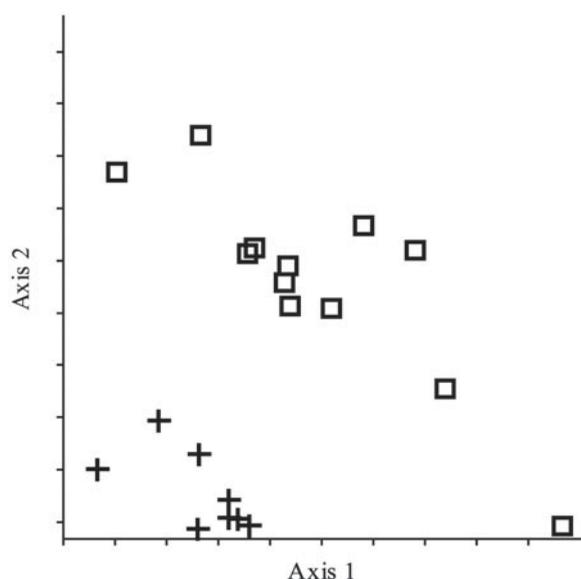


Fig. 2. Host shift experiment: Non-metric multidimensional scaling (NMDS; Bray-Curtis similarity measure) of the CHC profiles of *L. distinguendus* females. Hosts: □, *S. granarius* (BerSit; F1); +, *S. paniceum* (formerly BerSit; reared for one generation on *S. paniceum*; F1).

Cephalonomia hyalinipennis and the pteromalid wasp *Pteromalus cerealellae*, whereas the relative quantities of the components differed (Howard, 2001; Howard &

Perez-Lachaud, 2002). The biosynthesis of CHCs is closely associated with the fatty acid metabolism. For the synthesis of methyl-branched compounds, considerable amounts of valine, leucine, isoleucine, and methionine are also needed (Blomquist, 2010). These amino acids are among the essential dietary resources for insects, which cannot be biosynthesized *de novo* by themselves (Behmer, 2006). Hence, differing pools of limiting primary nutrients provided by the different hosts might account for the observed differences in the CHC profiles.

Interestingly, the CHC profiles of *L. distinguendus* wasps reared on *A. obtectus* and *S. granarius* overlapped in NMDS analysis, and the CHC profiles of wasps reared on *L. serricornis* cluster close to this group and are well separated from the CHC profiles of wasps reared on *S. paniceum*. Thus, the CHC profiles of wasps from these non-related hosts are more similar than the CHC profiles of those reared on *L. serricornis* and *S. paniceum*, which belong to the same family. It is most likely that *S. granarius*, *A. obtectus* and *L. serricornis* represent similar food substrates and provide qualitative and quantitative similar precursors for the CHCs of *L. distinguendus*, despite their phylogenetic differences.

In conclusion, our data demonstrate that the composition of CHC profiles in parasitic wasps depend on the host on which the wasps have developed. Because these differences arise already within one generation on a specific host, the composition of the CHC profiles is most likely determined by host-dependent precursors in the diet of the wasps. Since CHCs are known to play an important role in the recognition of conspecifics and mating partners in these insects (Sullivan, 2002; Steiner *et al.*, 2005, 2006, 2007; Ruther, *et al.*, 2011), it is

possible that the differences in CHC profiles caused by different hosts represent a reproductive barrier and may finally contribute to the formation of host-races and eventually to new species. This scenario might be more common in parasitic wasps, which could explain the high diversity in this group of insects. Future studies will have to address the question if the observed effects on the cuticular chemistry actually influence the courtship behaviour of *L. distinguendus*. Furthermore, it will be interesting to study which differences in host chemistry are responsible for the differences in the CHC profiles of *L. distinguendus*.

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Text summary of Supplementary Material

The supplementary material (pdf, 455 KB) consists of four figures showing comparative GC-MS chromatograms of cuticular extracts from female *Lariophagus distinguendus* wasps and the respective hosts (adult and larval stage).

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