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Striking cuticular hydrocarbon dimorphism in the mason wasp *Odynerus spinipes* and its possible evolutionary cause (Hymenoptera: Chrysididae, Vespidae)

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Cleptoparasitic wasps and bees smuggle their eggs into the nest of a host organism. Here the larvae of the cleptoparasite feed upon the food provision intended for the offspring of the host. As cleptoparasitism incurs a loss of fitness for the host organism (offspring of the host fail to develop), hosts of cleptoparasites are expected to exploit cues that alert them to potential cleptoparasite infestation. Cuticular hydrocarbons (CHCs) could serve as such cues, as insects inevitably leave traces of them behind when entering a nest. By mimicking the host's CHC profile, cleptoparasites can conceal their presence and evade detection by their host. Previous studies have provided evidence of cleptoparasites mimicking their host's CHC profile. However, the impact of this strategy on the evolution of the host's CHC profile has remained unexplored. Here, we present results from our investigation of a host–cleptoparasite system consisting of a single mason wasp species that serves syntopically as the host to three cuckoo wasp species. We found that the spiny mason wasp (*Odynerus spinipes*) is able to express two substantially different CHC profiles, each of which is seemingly mimicked by a cleptoparasitic cuckoo wasp (i.e. *Chrysis mediata* and *Pseudospinolia neglecta*). The CHC profile of the third cuckoo wasp (*Chrysis viridula*), a species not expected to benefit from mimicking its host's CHC profile because of its particular oviposition strategy, differs from the two CHC profiles of its host. Our results corroborate the idea that the similarity of the CHC profiles between cleptoparasitic cuckoo wasps and their hosts are the result of chemical mimicry. They further suggest that cleptoparasites may represent a hitherto unappreciated force that drives the evolution of their hosts' CHCs.

1. Introduction

Cleptoparasitic wasps and bees (Insecta: Hymenoptera) co-opt the maternal care of other species for the benefit of their own offspring. Cleptoparasites exploit their hosts by laying their own eggs, undetected, in the nest of the host. Subsequently, the larva of the cleptoparasite consumes the brood provisions intended for the

larva of the host. As the egg or larva of the host is killed or severely impaired, either by the ovipositing cleptoparasite or its developing offspring, cleptoparasites decrease the reproductive success and fitness of their hosts. Host species can mitigate the deleterious effects of cleptoparasitism by detecting furtive cleptoparasite attack and responding through aggressive defence or abandonment of the nest [1–3]. Accordingly, host vigilance and defence can lead to a decrease of the reproductive success and fitness of the cleptoparasite. Conflict between the two species thus drives the evolution of parasite detection in the host and host evasion in the cleptoparasite. Indeed, we expect an evolutionary arms race to occur in which hosts continuously evolve better detection strategies and cleptoparasites evolves better evasion strategies to counteract this. For further reading on cleptoparasitism and varying views of the terminology involved, we refer readers to the excellent reviews given by Eggleton & Belshaw [4] and Breed *et al*. [5].

Host organisms can maintain their fitness by detecting attempts of cleptoparasitism by exploiting sensory cues. Cues may be auditory, visual, or olfactory. Auditory and visual cues, however, are ineffective while the host is absent from the nest (e.g. during provisioning flights). Given that insects are covered by a layer of cuticular hydrocarbons (CHCs) that typically differ between species [6] and are inevitably left behind as traces when an insect enters a nest [2,3,7,8], detection of cleptoparasitism in insects can be achieved by means of exploiting olfactory cues. CHCs serve primarily as a desiccation barrier but have secondarily gained importance as cues and signals for intra- and interspecific recognition and communication. Cleptoparasites can evade olfactory detection by either applying olfactory cues collected from the host nest onto their own cuticle (i.e. by camouflage) or by evolutionary change of their own CHC profile [9,10]. The latter can be achieved by reducing the total amount of hydrocarbons on the cuticle (i.e. by applying an insignificance strategy) and/or by mimicking the CHC profile of the host (i.e. by applying a chemical mimicry strategy) [7,11].

The composition of CHCs in insects is largely species-specific and thus can serve as a reliable template for chemical mimicry. Previous studies on cleptoparasites mimicking the CHC profile of their hosts have mainly focused on host–cleptoparasite species pairs that are phylogenetically closely related [10]. Closely related species, however, already share a similar CHC profile and use similar pathways to biosynthesize CHCs inherited from their common ancestor (but see [6]). However, the vast majority of known cleptoparasites parasitize distantly related hosts (e.g. all cleptoparasitic cuckoo wasps; Hymenoptera: Chrysididae [12]). There are previously described instances, in which CHC profiles of hosts and CHC profiles of distantly related parasites proved to be strikingly similar. The explanation of this CHC profile similarity was camouflage: the physical acquisition of the host's CHCs by the parasite rather than biochemical synthesis. In that context, the phylogenetic distance between host and parasite (or parasitoid/cleptoparasite) is largely irrelevant [13,14]. An intriguing question remains: are cleptoparasites capable of imitating the CHC profiles of their phylogenetically distant hosts by true chemical mimicry (i.e. by evolving a CHC composition that corresponds to that of their host).

Strohm *et al*. [2,3] analysed and compared the CHC profiles of a digger wasp, *Philanthus triangulum* (Fabricius, 1775) (Insecta: Hymenoptera: Apoidea: Crabronidae), and its cleptoparasite, the cuckoo wasp *Hedychrum rutilans* Dahlbom, 1854 (Insecta: Hymenoptera: Chrysidoidea: Chrysididae). Despite the tremendous phylogenetic distance between host and cleptoparasite (their last common ancestor is thought to have lived in the Middle Jurassic, 170 Ma [15]), the authors found a striking similarity in the CHC profiles of both species. Strohm *et al*. [2,3] also analysed CHC profiles of species that are comparatively closely related to *P. triangulum* and *H. rutilans*, respectively, and that occur in the same habitat as these two species. However, the CHC profiles of those species largely differed from the CHC profiles of *P. triangulum* and *H. rutilans*. The authors, therefore, concluded that the CHC profile similarity of *H. rutilans* and *P. triangulum* is most probably the result of chemical mimicry. Studying CHC profile evolution in a complex host–cleptoparasite system, in which a single host species is attacked by multiple distantly related cleptoparasites, each of which benefits to varying degrees from chemically mimicking the CHC profile of their host, could provide further insights into the significance of chemical mimicry for cleptoparasites. It could furthermore shed light on what different solutions cleptoparasites have found for solving the same problem, i.e. evading olfactory detection by their host. Finally, it could indicate to what extent the cleptoparasites' action may also impact the evolution of the host's CHC profile.

A host–parasite system sufficiently complex to adequately address the above questions is that of the spiny mason wasp, *Odynerus spinipes* (Linnaeus, 1758) (Hymenoptera: Vespidae: Eumeninae), two cleptoparasitic cuckoo wasps, *Chrysis mediata* Linsenmaier, 1951 and *Pseudospinolia neglecta* (Shuckard, 1836) (Hymenoptera: Chrysididae: Chrysidinae), and the parasitoid cuckoo wasp *Chrysis viridula* Linnaeus, 1761. The spiny mason wasp, *O. spinipes*, builds its nest in the ground and prefers vertical surfaces of fine sediment, such as sand and loess. *O. spinipes* females burrow nests with one entrance each that protrudes from the surface as a funnel-like tube of soil and remains open until all brood cells have been provisioned with prey. The prey consists exclusively of beetle larvae of the genus *Hypera* (Curculionidae). When a brood cell has been stocked, the *O. spinipes* female lays an egg in it, closes the cell and moves to build and provision the next cell. Once all brood cells are finished, the female seals its nest entrance with a plug made of soil [16].

The cuckoo wasps that parasitize nests of *O. spinipes* apply different strategies to intrude their host's nests and to lay eggs into the brood cells [17–19]. The parasitoid cuckoo wasp *C. viridula* opens already sealed *O. spinipes* nests for oviposition and places an egg next to a diapausing *O. spinipes* prepuae, which the developing parasitoid cuckoo wasp larva subsequently feeds on. By contrast, *C. mediata* and *P. neglecta* enter their host's nest while the nest is still open. Specifically, they enter the host's nests during the short time periods when *O. spinipes* females are searching for prey or nest material. It follows that *C. mediata* and *P. neglecta* could probably increase their reproductive success by chemically mimicking the CHC profile of *O. spinipes* females, thereby obfuscating their nest intrusion from the host, while *C. viridula* does not need to do so, as *O. spinipes* does not guard its sealed nests. Despite using the same host and, in the case of *C. mediata* and *P. neglecta*, even applying the same nest intrusion strategy, the three cuckoo wasps are only distantly related [20]. This suggests that *O. spinipes* probably became the host of cuckoo wasps at least three times independently. In this study, we seek to exploit the *O. spinipes* host–parasite system to assess whether some cleptoparasitic cuckoo wasps indeed apply a chemical mimicry strategy and, if so, how it may impact the evolution of their

host's CHC profile. Specifically, we (i) characterize the CHC profiles of males and females of *O. spinipes* and of females of *C. mediata*, *C. viridula*, and *P. neglecta* qualitatively and quantitatively. For outgroup comparison, we additionally characterize the CHC profile of females of *Odynerus reniformis* (Gmelin, 1790), which in some places occurs syntopically with *O. spinipes* and which probably does not serve as host of the above three cuckoo wasp species. We (ii) compare the CHC profiles of the three cuckoo wasps with each other and with those of the host, *O. spinipes*. This allows us to assess whether or not the two cleptoparasitic cuckoo wasps that should benefit from chemically mimicking the CHC profile of *O. spinipes* females (i.e. *C. mediata* and *P. neglecta*) share a higher CHC profile similarity with *O. spinipes* females than *C. viridula* females do. As all three cuckoo wasps develop in brood cells of the same host species, this comparison allows us to disentangle CHC profile similarity because of microclimatic adaptation from CHC profile similarity because of chemical mimicry. We corroborate our conclusions by (iii) comparing the CHC profiles of the three cuckoo wasp species with those of *O. reniformis*. We hypothesize that CHC profiles of females of *C. mediata* and *P. neglecta* are more similar to the CHC profile of females of their host than to that of females of *O. reniformis*. Finally, we (iv) compare CHC profiles of females of *C. mediata*, *C. viridula*, *P. neglecta*, and *O. spinipes* collected at two distant field sites to determine possible signs of local shifts in the species' CHC profiles.

2. Material and methods

(a) Samples and field sites

For chemical analyses mason and cuckoo wasps were collected at three *Odynerus* nest aggregations. All wasps were freeze-killed on the day of collection and were stored at −20°C. After thawing and extraction of their CHCs, the wasps were transferred to 96% ethanol for subsequent DNA analyses. For detailed information on sample times and sizes, see the electronic supplementary material, S12.

(b) Chemical analyses of cuticular hydrocarbon profiles

CHCs were extracted by submerging the freeze-killed wasps for 10 min in n-pentane. In all extracts, the volume of n-pentane was adjusted to 200 µl, and all extracts were stored at −20°C prior to chemical analysis. The CHC extracts were analysed with a gas chromatograph coupled with a mass selective detector. For detailed information on the specific configurations and processes used, see the electronic supplementary material, S12. All CHC compositions have been deposited in a Dryad Data Repository at http://dx.doi.org/10.5061/dryad.1d63s.

(c) Comparison and statistical analyses of cuticular hydrocarbon profiles

We assessed CHC profile similarity by means of multivariate methods, i.e. non-metric multidimensional scaling (NMDS) and an agglomerative hierarchical cluster analysis. The spatial distances between points in the NMDS plot indicate the chemical differences between samples, and the corresponding stress value indicates the goodness of fit of the two-dimensional representation to the initial multidimensional distances [21,22]. The differences in Bray–Curtis distances between species pairs were assessed by Welch's two sample *t*-test using the *t*-test function of the stats package in R [23]. Cluster distances for the agglomerative cluster analysis were calculated using the complete linkage method. For detailed information on methods and functions used, see the electronic supplementary material, S12.

Note that neither hierarchical clustering nor NMDS require *a priori* knowledge of what samples probably belong to the same group. Any data structures emerging from these methods are solely based on similarities of the chemical compositions of the analysed extracts.

(d) Comparative DNA analyses

To assess whether the high differentiation that we found among the CHC profiles of *O. spinipes* females (see below) is mirrored by conspicuous differentiation of the species at the genomic level, we barcoded 17 females of *O. spinipes*, of which 12 exhibited CHC chemotype 1 and five CHC chemotype 2, by sequencing a major part of their mitochondrial gene Cytochrome-*c*-Oxidase I (COI). For detailed information on the specific extraction and amplification processes used, see the electronic supplementary material, S12.

The chromatograms of the obtained nucleotide sequences were analysed and assembled to contigs with GENEIOUS 5.3 (Biomatters, Auckland, New Zealand). We used the same software to align the nucleotide sequences of the different samples to each other. All sequences are deposited in the EMBL nucleotide archive (accession nos. HG316086-HG316103).

(e) Morphometric analyses

To assess whether the high differentiation that we found among the CHC profiles of *O. spinipes* females is possibly also mirrored in a differentiation of morphometric traits, we conducted a morphometric analysis. For this purpose, we analysed a subset of 53 dry-mounted *O. spinipes* females. Biometry specimens have been deposited in the ZFMK BioBank archive (accession nos. ZFMK-HYM-00010000–ZFMK-HYM-00010074).

We quantified 26 morphological characters (electronic supplementary material, table S8) and 13 forewing landmarks (electronic supplementary material, figure S3) for each specimen. Multivariate ratio analysis (MRA) [24] was used for analysing distance measurements, while generalized least-squares Procrustes analysis was applied to the landmarks using the program MorphoJ [25]. We also assessed the measurement error (see electronic supplementary material, table S9), because it may have a devastating effect on multivariate analyses and obscure effects in the data (see [26]). For detailed information, the specific hard and software as well as the workflow and scripts used for imaging and measurements, see the electronic supplementary material, S12 and S13. All measurements and coordinates have been deposited in a Dryad Data Repository at http://dx.doi.org/10.5061/dryad.1d63s.

3. Results

The CHC extracts of the investigated species revealed species- and gender-specific CHC compositions. All CHC profiles consisted primarily of alkanes, alkenes, and monomethyl-branched alkanes. Alkadienes, alkatrienes, and dimethyl-branched alkanes were much less abundant in the analysed CHC extracts (electronic supplementary material, tables S5 and S10).

The CHC profiles of the investigated females of *O. spinipes* fall into two very distinct groups, which we hereafter refer to as chemotype 1 and chemotype 2 (see also results of NMDS analysis below; figures 1 and 2; electronic supplementary material, figures S1 and S2). The observed qualitative differences between the two chemotypes of *O. spinipes* females are well within the range of differences between the CHC profiles of distinct species (electronic supplementary material, table S5; figure 1). All males of *O. spinipes* exhibit very similar CHC profiles that strongly resemble those of females expressing chemotype 1.

Given that a cleptoparasite that is chemically mimicking its host should seek to minimize its overall amount of diagnostic CHC compounds (i.e. compounds exclusive to the cleptoparasite

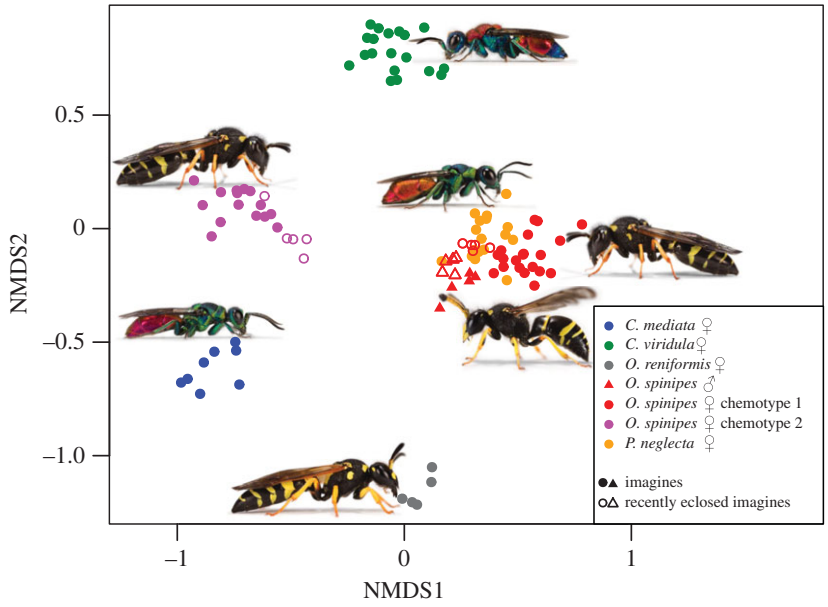

Figure 1. Similarity of cuticular hydrocarbon (CHC) profiles of females of *Chrysis mediata* ($n = 8$), *Chrysis viridula* ($n = 19$), *Odynerus reniformis* ($n = 5$), males of *Odynerus spinipes* ($n = 10$), females of *Odynerus spinipes* chemotype 1 ($n = 25$) and chemotype 2 ($n = 20$), respectively, and *Pseudospinolia neglecta* ($n = 17$) displayed in a two-dimensional graph by non-metric multidimensional scaling (NMDS) of Bray–Curtis CHC profile distances (spatial proximity correlates with high CHC profile similarity, stress = 0.16). All photographs by O. Niehuis.

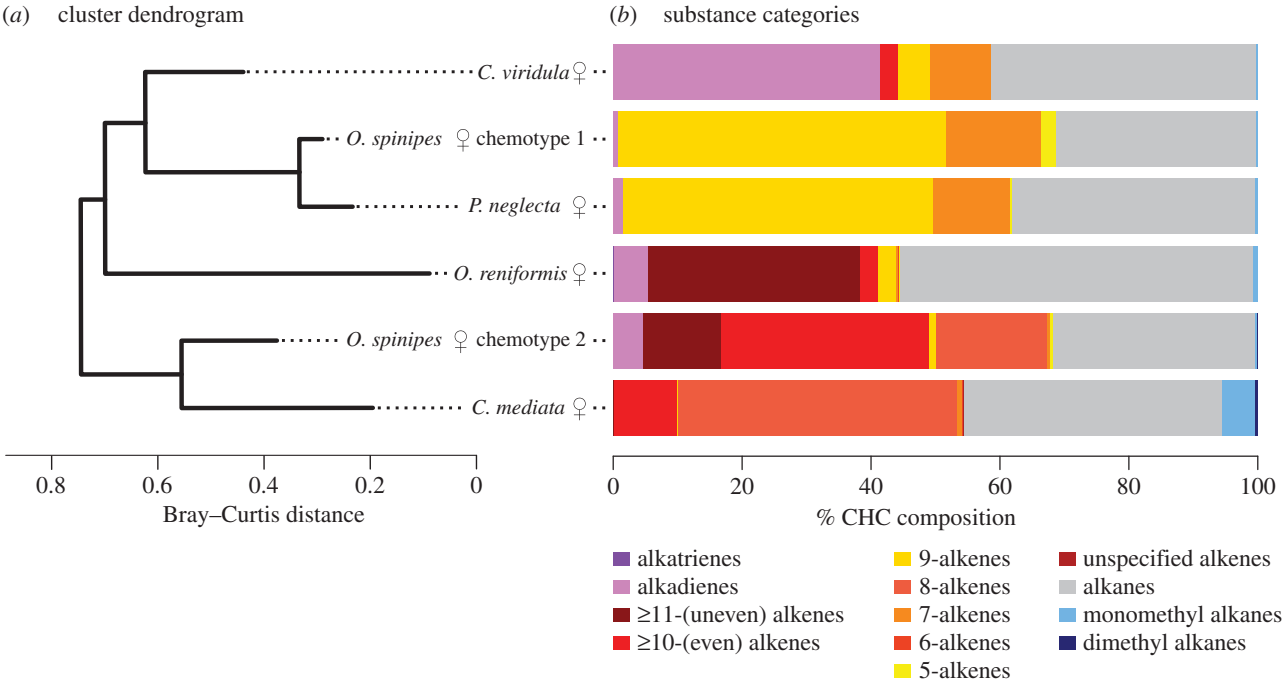

Figure 2. (*a*) Similarity of cuticular hydrocarbon (CHC) profiles of females of *Chrysis mediata* ($n = 8$), *Chrysis viridula* ($n = 19$), *Odynerus reniformis* ($n = 5$), *Odynerus spinipes* chemotype 1 ($n = 20$), and chemotype 2 ($n = 15$), respectively, and *Pseudospinolia neglecta* ($n = 17$) displayed in a cluster analysis dendrogram of Bray–Curtis CHC profile distances (see also electronic supplementary material, table S11); note that endpoints of branches vary between species/chemotypes because of different within-group variation. (*b*) Average relative proportion (in per cent) of major hydrocarbon substance classes in the CHC profiles of the same individuals as in (*a*).

when compared with its host), we also calculated the total amount of qualitatively diagnostic CHC compounds in the average CHC profile of each cuckoo wasp relative to the two chemotypes of *O. spinipes* females. When compared with chemotype 1, *C. mediata* females possess 42 diagnostic CHC compounds, *C. viridula* females possess 46, and *P. neglecta* females possess 15. When compared with chemotype 2, *C. mediata* females possess 24 diagnostic CHC compounds, while *C. viridula* possess 39 and *P. neglecta* possess 25, (electronic supplementary material, table S6).

COI nucleotide sequence comparisons among females of *O. spinipes* revealed no systematic difference in the mitochondrial haplotypes of females belonging to chemotype 1 and those belonging to chemotype 2: uncorrected *p* distances within and between the two groups were 0.0–0.3% (chemotype 1), 0.0–0.3% (chemotype 2), and 0.0–0.3% (between chemotypes), respectively.

MRA of body measurements and Procrustes analysis of 13 landmarks on the forewing revealed no differences between the two chemotypes, neither in size nor in shape axes (figure 3 and electronic supplementary material, figure S4). The inferred scatterplots are in accordance with the significance tests: univariate tests for mean differences in isometric and centroid size had $p > 0.8$, while multivariate tests for mean shape differences in distance

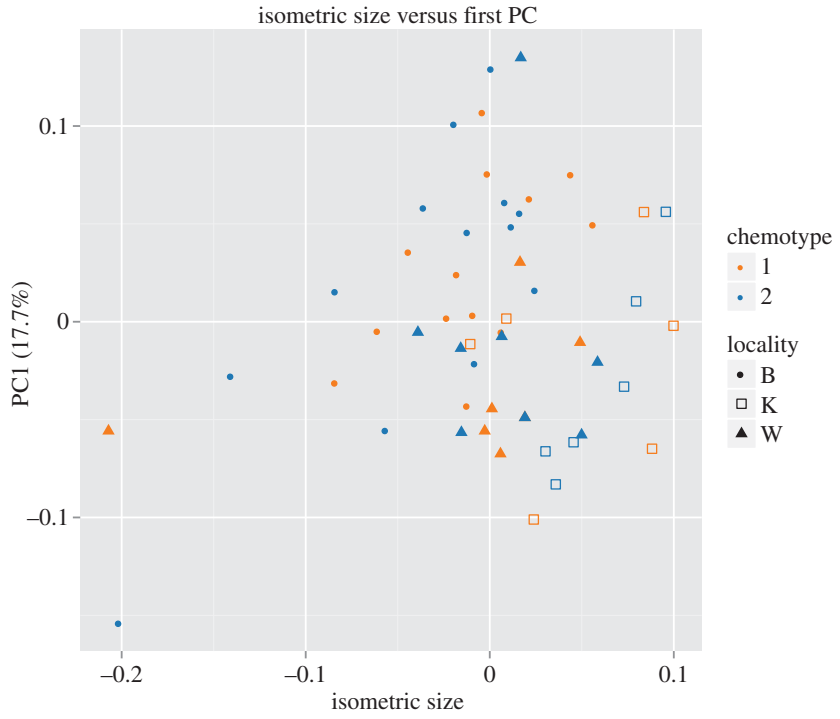

Figure 3. Morphometric analysis of *Odynerus spinipes* females. Multivariate ratio analysis (MRA) of distance measurements, scatterplot of isometric size versus first shape PC. Colour: orange, chemotype 1, blue, chemotype 2. Shapes: dots, Büchelberg; open squares, Kaiserstuhl, Oberbergen; triangles, Würzburg, Hubland. In parentheses, the variance explained by each shape PC.

and landmark data had $p > 0.5$. Figure 3 indicates that specimens from Kaiserstuhl are on average larger than specimens from other locations. They also seem to differ slightly in shape (mainly on the second shape PC). For all tested distance measurements, the measurement error was below the critical level of 30% (electronic supplementary material, table S9).

Qualitative differences in the CHC profiles of the analysed species and chemotypes are most pronounced in alkenes (figure 2; electronic supplementary material, figure S2 and table S10). The CHC profiles of male *O. spinipes*, *O. spinipes* females belonging to chemotype 1, and females of *P. neglecta* are characterized by a dominance of alkenes with double bonds at positions 5, 7, and 9 (electronic supplementary material, figures S1, S2A and B). By contrast, the CHC profiles of females of *O. spinipes* belonging to chemotype 2 are characterized by alkenes with double bonds at positions 8, 10, 12, and 14 (electronic supplementary material, figures S2A and C). The CHC profiles of females of *C. mediata* are characterized by a dominance of alkenes with double bonds at positions 8 and 10 (electronic supplementary material, figure S2C), and those of C. *viridula* by alkenes with double bonds at positions 7 and 9 (electronic supplementary material, figure S2B). Finally, the CHC profiles of females of *O. reniformis* mostly exhibit alkenes with double bonds at positions 9, 11, 13, and 15 (electronic supplementary material, table S10). Note, however, that chemical similarities are not simply a list of common and exclusive substances but are modulated by quantitative aspects, i.e. the ratios of substances within the specific CHC mixtures. The subsequent analyses (NMDS and cluster analysis) took both qualitative and quantitative measures into account.

CHC profile composition analysed by NMDS (figure 1, stress: 0.16) corroborates our intuitive *ad hoc* clustering of the analysed samples into six groups. The CHC profiles of females of the species *O. reniformis*, *C. viridula*, *C. mediata*, and *P. neglecta* each comprise species-specific groups. By contrast, the CHC profiles of females of *O. spinipes* comprise two distinct groups that correspond to the aforementioned two chemotypes. Males of *O. spinipes* exhibit only one CHC profile that is similar to that of the female chemotype 1 and contains the identical set of alkene isomers. Freshly eclosed *O. spinipes* do not differ from older mated individuals, and freshly eclosed females are (as with the older nest-building females) capable of expressing both chemotypes.

The occurrence of the two *O. spinipes* chemotypes does not correlate with sampling locations and years. In fact, both chemotypes have been collected simultaneously in all three locations (each > 100 km beeline distance from either other; in Büchelberg in 2013 and 2014, in Oberbergen in 2012, and in Würzburg in 2007 and 2011).

The NMDS graph indicates that the CHC profiles of *P. neglecta* females strikingly resemble, in their qualitative and quantitative chemical composition, those of *O. spinipes* females belonging to chemotype 1. The CHC profiles of *C. mediata* females resemble those of *O. spinipes* females belonging to chemotype 2, although to a lesser extent. CHC profiles of females of *C. viridula*, by contrast, exhibit no major similarity with either of the two *O. spinipes* chemotypes. Note that CHC profiles of none of the three cleptoparasites show noteworthy similarity with the CHC profiles of *O. reniformis*. A hierarchical cluster analysis based on Bray–Curtis distances of the CHC profiles corroborates this result (figure 2*a*; electronic supplementary material, table S11). The similarity between the CHC profiles of *P. neglecta* females and those of *O. spinipes* females belonging to chemotype 1 on the one hand, and between the CHC profiles of *C. mediata* females and those of *O. spinipes* females belonging to chemotype 2, on the other hand, becomes particularly evident when looking at the average relative abundance of individual substance categories. Examples are alkenes with a double bond at a specific position and methyl-branched alkanes with the branching point at a given carbon atom (figure 2*b*; electronic supplementary material, figures S1, S2, and table S10).

4. Discussion

(a) *Odynerus spinipes* cuticular hydrocarbon profile divergence

CHCs are generally thought to be species-specific and to differ little among individuals and populations of a given species,

even over large geographical distances [27,28]. The narrow clustering of the CHC profiles belonging to a given species in the NMDS graph (figure 1) corroborates this assumption with the notable exception of the CHC profiles of *O. spinipes* females. The fact that the CHC profiles of females of *O. spinipes* fall into two quantitatively and qualitatively very distinct groups or chemotypes (figures 1 and 2; electronic supplementary material, figures S1, S2, and table S10) was unexpected. The two chemotypes cannot readily be interpreted as two extremes of an insufficiently sampled broader spectrum of variation as, despite substantial sampling, no intermediate CHC profiles were identified. We also exclude local adaptation as a possible explanation for the occurrence of two distinct chemotypes as both chemotypes were found at the same time and the same locality. Moreover, we found that this pattern was mirrored at distant locations and over multiple field seasons. Neither do the two chemotypes represent the sexes of the wasp as both chemotypes are found in females nor do they reflect age classes or mating status as both chemotypes are found in freshly eclosed virgin females and persist over the entire duration of the flight season.

The differences between the two CHC chemotypes of *O. spinipes* females are particularly evident when looking at alkene isomers (figure 2*b*; electronic supplementary material, figure S2A): the alkenes in the CHC profile of *O. spinipes* females belonging to chemotype 1 exhibit primarily a double bond at carbon atom 5, 7, or 9. Alkenes in the CHC profile of females belonging to chemotype 2, by contrast, exhibit primarily double bonds at carbon atoms with an even number (8–14) (electronic supplementary material, table S10). The occurrence of two chemotypes that differ consistently in their alkene isomer composition (i.e. one chemotype is characterized by compounds with double bonds at even positions, the other by compounds with double bonds at odd positions) is probably the result of a major chemical shift (see [29], for an example of such a shift between sister species), and this shift presumably corresponds to a differentiation in biosynthetic pathways (see below [30]).

Alkenes are known to play an important role in various recognition systems as species-specific pheromones (e.g. in leafcutter bees, Hymenoptera: Megachilidae [31]; tsetse flies, Diptera: Glossinidae [32]; longhorn beetles, Coleoptera: Cerambycidae [33]) and for nest-mate recognition (e.g. social wasps, ants, and bees, Hymenoptera: Vespidae, Formicidae, Apidae [34]). Alkene isomers, in particular, account for the species-specificity of CHC profiles in bumblebees [35]. Distinct chemotypes within species have been found so far only in allopatric populations of fruit flies (Diptera: Drosophilidae [36]) and bumblebees (Hymenoptera: Apidae [35]), and in sympatric individuals of termites (Isoptera: Termitidae [37]) and digger wasps (Hymenoptera: Crabronidae [2,3]). However, except for the above-mentioned CHC profile polymorphism within termites, all distinct intraspecific chemotype differences recorded so far are only quantitative in nature, while the chemotypes of *O. spinipes* females are largely because of qualitative differences.

The qualitative differences between the *O. spinipes* chemotypes are manifested in the production of alkene isomers with either uneven- or even-numbered double bond positions. In insects, various genes encoding fatty acid desaturases have been described. However, only three of these genes are explicitly known to be involved in hydrocarbon biosynthesis (exclusively in species of *Drosophila*), all of which exhibit Δ9 specificity [38]. Desaturases with other specificities (e.g. Δ10 and Δ14) have been described in Lepidoptera [39,40], but are expected to occur in other taxa, too [38]. Roelofs *et al.* [40] discussed how the activation of a previously dormant pseudogene coding for a Δ14 desaturase could have given rise to a new pheromone blend and, ultimately, to a new species in Lepidoptera. A similar mechanism could, at least in theory, have given rise to the ability to biosynthesize two different CHC profiles in *O. spinipes* females without going through a speciation process.

One possible explanation for the two distinct chemotypes in female *O. spinipes* is the presence of a second, cryptic, species that coexists syntopically with *O. spinipes*. The observation of similar patterns in ants [41] and termites [42] has indeed led to the discovery of previously unrecognized species. In all these cases, however, the distinct chemotypes correlated with distinct genetic lineages. In *O. spinipes*, COI nucleotide sequences do not provide evidence for the presence of distinct genetic lineages that correlate with the two distinct chemotypes. It must be emphasized, however, that the mitochondrial gene COI is exclusively maternally inherited and can only serve as a proxy for identifying two reproductively isolated species. This is because recent introgression of the mitochondrial genome of one of two such species into the other species, perhaps promoted by *Wolbachia* bacteria, which are common in Hymenoptera, and also maternally transmitted, could result in largely uniform mitochondrial haplotypes despite genetic distinctness in the nuclear genome. However, we did not find any colour trait nor any morphometric characteristics (figure 3) that correlate with the two distinct chemotypes expressed by female *O. spinipes*. Given that both colour traits and morphological characters are largely, if not entirely, encoded by nuclear genes, these results corroborate the assumption that individuals of different chemotype belong to the same species.

We therefore conclude that the two chemotypes of *O. spinipes* do not reflect cryptic species and instead hypothesize that the two chemotypes are the result of an evolutionary arms race between *O. spinipes* females and females of one or both of its cleptoparasites (see below). We assume that, over the course of this arms race, *O. spinipes* females evolved the ability to express two different CHC profiles. By doing so, a given cleptoparasite would only be able to mimic the CHC profiles of those females in a *O. spinipes* population whose chemotype matches the cleptoparasite's CHCs. Females of *O. spinipes* exhibiting the alternative chemotype should consequently suffer less from this cleptoparasite. In eusocial species that serve as hosts of social parasites, the diversification of a species-specific profile into colony-specific chemotypes has been interpreted as a strategy to counteract parasites that apply a chemical mimicry strategy [37,43]. It must be emphasized, however, that the presence of two alternative chemotypes is probably evolutionarily stable only under balancing selection, i.e. if the second chemotype is also mimicked by a cleptoparasite. This evolutionary scenario is probably realized in the *O. spinipes* host–parasite system, with *O. spinipes* being parasitized by two species (i.e. *C. mediata* and *P. neglecta*) that each seem to mimic a different *O. spinipes* chemotype (see below).

(b) Evidence for cleptoparasites mimicking the cuticular hydrocarbon profile of their host

The data obtained from analysing the CHC profiles of *O. spinipes*, *C. mediata*, and *P. neglecta* in combination with

those of *C. viridula* and *O. reniformis* strongly suggests that females of *P. neglecta* chemically mimic the CHC profile of *O. spinipes* females belonging to chemotype 1 both qualitatively and quantitatively. Likewise, females of *C. mediata* seem to chemically mimic females of *O. spinipes* belonging to chemotype 2. However, the similarity in the CHC profiles of *C. mediata* and *O. spinipes* females exhibiting chemotype 2 is mostly qualitative (i.e. the two species synthesize largely the same CHC compounds, but the abundance of these compounds differ between the two species). Nonetheless, in both host–parasite species pairs (i.e. *O. spinipes* chemotype 1 and *P. neglecta*, and *O. spinipes* chemotype 2 and *C. mediata*), Bray–Curtis distances between the CHC profiles of the cleptoparasites and those of their respective host are significantly smaller than the distances between CHC profiles of these two cleptoparasites and the CHC profiles of the non-mimicked chemotype (Welch's two sample *t*-test, for *P. neglecta*: $T = -48.68$, d.f. $= 748$, $p < 0.01$, for *C. mediata*: $T = 22.61$, d.f. $= 327$, $p < 0.01$).

The high similarity between the CHC profiles of *O. spinipes* and its cleptoparasites cannot be explained by physical coating of the cleptoparasites with the host's CHCs (i.e. by chemical camouflage) because only a subset of the host's CHC compounds are also found in the CHC profile of the cleptoparasites. Specifically, *O. spinipes* chemotype 1 possesses 35 substances not found on the cuticle of *P. neglecta*. Likewise, *O. spinipes* chemotype 2 possesses 47 substances not found on the cuticle of *C. mediata*. These observations are incompatible with a chemical camouflage strategy, as cleptoparasites cannot selectively transfer substances from the host's cuticle or nesting material to their own cuticle [9]. Thus, the most likely explanation for the CHC profile similarity between females of *O. spinipes*, *P. neglecta*, and *C. mediata* is chemical mimicry (i.e. evolutionary change of the cleptoparasites' CHC biosynthesis to synthesize a CHC blend resembling that of the host).

Kroiss *et al*. [11] provided data suggesting that some cuckoo wasps may apply an insignificance strategy to evade chemical detection. Specifically, they showed that the total amount of CHCs on the surface of the cuckoo wasp *H. rutilans* is lower than on other non-cleptoparasitic hymenopterans. Thus, *H. rutilans* may also evade chemical detection by leaving only smaller amounts of CHCs behind than other, non-cleptoparasitic hymenopterans. We consequently also assessed the possibility that the two cleptoparasites of *O. spinipes* apply a chemical insignificance strategy by following the procedure outlined by Kroiss *et al*. (i.e. by relating the extracted amount of CHCs with the estimated body surface area; electronic supplementary material, table S7). However, while Kroiss *et al*. [11] found an estimated 10-fold reduction of the total amount of CHCs per body surface of *H. rutilans* relative to their host, we found no conspicuous differences of CHC amounts per body surface between *O. spinipes* and its cleptoparasites.

The extent by which the CHC profiles of *C. mediata* and *P. neglecta* match their respective host CHC chemotype of *O. spinipes* differs. The CHC profiles of females of *P. neglecta* exhibit both the same alkene isomers and a similar relative abundance of these isomers as those of the hypothesized models, i.e. females of *O. spinipes* belonging to chemotype 1. The CHC profiles of females of *C. mediata*, by contrast, exhibit the same dominant compounds as the CHC profiles of the hypothesized models, i.e. females of *O. spinipes* belonging to chemotype 2. However, relative abundance among the dominant compounds differs significantly in the latter species pair. It has to be stated, though, that a seemingly non-perfect match of relative abundance ratios of the dominant CHC compounds between a mimic and a model does not necessarily result in a less effective ability of the mimic/cleptoparasite to evade chemical detection by the model/host. In fact, we assume that the efficiency of chemical mimicry in cleptoparasitic cuckoo wasps depends on the mimic's ability to synthesize primarily or even exclusively chemical compounds that are part of the CHC profile of the host/model. The walls of the nest burrow might be covered by host CHC [11], thus making small amounts of relatively similar CHCs even less conspicuous than on a bare background. The mimic's CHC profile thereby probably becomes difficult to detect for the host, as it blends into the host's own chemical trails.

Consistent with the idea that CHC profile similarity between hosts and cleptoparasites that enter their hosts' nests is due to chemical mimicry and does not reflect a microclimatic adaptation is the observation that females of *C. viridula* seemingly do not mimic the CHC profiles of their host. As mentioned before, *C. viridula* differs in its oviposition strategy from *C. mediata* and *P. neglecta* by re-opening the sealed nests of its host. Hence, there is no need for *C. viridula* females to chemically mimic the CHC profile of their host. Our results thus contradict the hypothesis that CHC profile similarity between *O. spinipes* and its cleptoparasites is the result of adaptations to a similar microclimate or other abiotic factors, because the parasitoid *C. viridula* develops in the same nests as the cleptoparasites *C. mediata* and *P. neglecta*.

Our data contradict the hypothesis that hosts and their cleptoparasites share similar CHC profiles because they share the same diet. The hypothesis is derived from the idea that specific metabolites that are part of an organism's diet could serve as precursor substances required for the biosynthesis of CHCs (as shown, e.g. in ants by [44]). If diet had a major impact on the CHC profile, we would not expect the two cleptoparasites to drastically differ in their CHC profiles, as they both feed on *Hypera* larvae, as do the larvae of *O. spinipes*. While there is the possibility that *O. spinipes* females with different chemotypes prey on different *Hypera* species, a dietary impact on the CHC profile of *Odynerus* should then also be mirrored in males. This is clearly not the case, as all *O. spinipes* males share similar CHC profiles. The fact that the CHC composition of males closely resembles that of females exhibiting chemotype 1 can have various reasons that future studies might be able to assess. Given that we studied the CHC profiles of a large number of males it is unlikely that we simply missed a second male chemotype by chance. If the striking difference in the CHC profiles of *O. spinipes* females is the result of an evolutionary arms race between a host and at least two cleptoparasites in which only the host female is and was able to counteract the chemical mimicry strategy of the cleptoparasites, males of the host species would probably never experience the selective forces that led to the evolution of CHC dimorphism in females. Future experiments focusing on the genetics of the striking CHC dimorphism in *O. spinipes* females and exploring the role of chemical cues in communication between *O. spinipes* males and females (e.g. during mate choice) have the potential to uncover the evolutionary causes for the astounding sex-specific CHC dimorphism in this species.

The three cuckoo wasps *C. mediata*, *C. viridula*, and *P. neglecta* have been reported to also exploit *O. reniformis* as a secondary host (reviewed in [45]). However, to the best of our knowledge, neither of these cuckoo wasps has ever been raised from *O. reniformis* nests. Considering that the match between CHC profiles of the two *C. mediata* and *P. neglecta* and of *O. reniformis* is so poor, compared with the matches between the cleptoparasites and *O. spinipes*, we conclude that *O. reniformis* has not been—at least in its recent past—host to either cleptoparasite. However, *C. viridula* does not need to chemically mimic the CHC profile of its host. Hence, the question of whether or not *O. reniformis* may serve as a host of this parasitoid cannot be inferred from the chemical data and remains to be investigated.

Data accessibility. All reported sequences have been deposited in the EMBL nucleotide archive (accession nos. HG316086–HG316103). Raw datasets of CHC compositions, distance measurements, and landmark coordinates have been deposited in a Dryad Data Repository at http://dx.doi.org/10.5061/dryad.1d63s. All biometry specimens have been deposited in the ZFMK BioBank archive (accession nos. ZFMK-HYM-00010000–ZFMK-HYM-00010074).

Authors' contributions. M.W. carried out sample collection, chemical analysis, statistical analysis of chemical data, morphometric measurements, participated in the design of the study, and drafted the manuscript; S.H. carried out the molecular laboratory work; D.D. helped with data interpretation; J.K. and E.S. participated in the design of the study; H.B. carried out statistical analysis of morphometric data; O.N. participated in the design of the study, supervised the molecular part of the study, and helped draft the manuscript; T.S. conceived the study, participated in the design of the study, and helped draft the manuscript. All authors gave final approval for publication.

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