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RESEARCH ARTICLE

Insect cuticular hydrocarbon composition influences their interaction with spider capture threads

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ABSTRACT

Insects represent the main prey of spiders, and spiders and insects co-diversified in evolutionary history. One of the main features characterizing spiders is their web as a trap to capture prey. Phylogenetically, the cribellate thread is one of the earliest thread types that was specialized to capture prey. In contrast to other capture threads, it lacks adhesive glue and consists of nanofibres, which do not only adhere to insects via van der Waals forces but also interact with the insects' cuticular hydrocarbon (CHC) layer, thus enhancing adhesion. The CHC layer consists of multiple hydrocarbon types and is highly diverse between species. In this study, we show that CHC interaction with cribellate capture threads is affected by CHC composition of the insect. We studied the interaction in detail for four insect species with different CHC profiles and observed a differential migration of CHCs into the thread. The migration depends on the molecular structure of the hydrocarbon types as well as their viscosity, influenced by the ambient temperature during the interaction. As a consequence, adhesion forces to CHC layers differ depending on their chemical composition. Our results match predictions based on biophysical properties of hydrocarbons, and show that cribellate spiders can exert selection pressure on the CHC composition of their insect prey.

KEY WORDS: Adhesion, Cribellate capture thread, Cuticular hydrocarbons, Evolutionary arms race, Predator–prey interactions, Selection pressure, Spider silk, Viscosity

INTRODUCTION

Spiders are important predators in almost all terrestrial habitats across the earth (Foelix, 2011). With insects representing over 90% of their prey, a tight co-evolution between spiders and insects can be assumed, with potential for an evolutionary arms race between spider predators and insect prey (Nyffeler and Birkhofer, 2017). For example, the proliferation of insects in the Late Cretaceous presumably also caused the proliferation and diversification of spiders found today (Garrison et al., 2016; Vollrath and Selden, 2007). The first specialized capture threads used by spiders were probably cribellate, compound threads of different silks with no glue but nanofibres as adhesive material (Joel et al., 2015; Friedrich and Langer, 1969). Several studies showed that spider capture threads interact with various surface features of the insects, such as hairs (Opell, 1994; Opell and Schwend, 2007). Only recently, it was discovered that the ability of cribellate threads to capture prey is not just due to their entangling in surface irregularities and van der Waals forces, which cause nanofibres to attach to any surface (Hawthorn and Opell, 2003). Much more importantly, cribellate threads also interact with the cuticular hydrocarbon (CHC) layer covering their prey, leading to an 8 times stronger attachment compared with CHC-free surfaces (Bott et al., 2017).

CHCs cover the body surface of nearly all insects. Their presumed original function was to reduce water loss, but they also serve other tasks such as interspecific and intraspecific communication (Ramsay, 1935; Wigglesworth, 1945; Menzel and Schmitt, 2012; Bos et al., 2011; Wüst and Menzel, 2017; Leonhardt et al., 2016). For these purposes, an individual's CHC layer consists of a complex blend of sometimes more than 100 different compounds (Blomquist and Bagnères, 2010). The profile is mainly genetically determined and species specific, i.e. members of a species usually possess the same set of hydrocarbons (Sprenger and Menzel, 2020; Blomquist and Bagnères, 2010), albeit many species show sexual CHC dimorphism (Jallon, 1984; Thomas and Simmons, 2008). Within the same species and sex, the quantitative composition can vary to some degree depending on, for example, climatic conditions or food (Liang and Silverman, 2000; Menzel et al., 2018; Sprenger et al., 2018; Woodrow et al., 2000; Wagner et al., 2001) The qualitative and quantitative composition influences the physico-chemical properties (e.g. viscosity and melting points) of the CHC coating, and thus its functionality (Blomquist and Bagnères, 2010; Menzel et al., 2019). For example, melting points increase with higher chain length (Gibbs and Pomonis, 1995). However, methyl branches and double bonds hinder tight packing of the hydrocarbon molecules, and thereby drastically reduce melting points (or, for liquid phases, viscosity). These effects are stronger than those of chain length, i.e. at the same chain length, a methyl group or a double bond can reduce the melting point of a hydrocarbon by 10–30 K (Gibbs and Pomonis, 1995; Gibbs, 2002), whereas the increase of chain length by one carbon atom only increases the melting point by ca. 2 K (Gibbs and Rajpurohit, 2010). CHC melting is biologically relevant because waterproofing is achieved mainly by solid or highly viscous components; at higher temperatures, insects therefore adjust their CHC composition to increase viscosity (Sprenger et al., 2018).

CHCs play an important role in insects and various selection pressures influencing its composition have been studied (Sprenger et al., 2018; Chung and Carroll, 2015; Menzel et al., 2017a; Menzel et al., 2017b; Kleeberg et al., 2017). Because the interaction between spider silk and insect CHCs has been unknown until recently, studies on potential selection pressures of spider predators on the insect CHC composition are missing. However, the efficiency of the cribellate capture mechanism largely depends on its interaction with the CHC layer. Hence, it was suggested that an evolutionary arms race might have favoured insects with more viscous CHC layers and, conversely, that capture threads bearing

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their own glue (i.e. viscous capture threads of Araneidae) evolved to escape the dependence on prey CHCs (Bott et al., 2017). A thread–CHC interaction (detectable in the scanning electron microscope) was described for 65% of the tested insects (Bott et al., 2017). It was hypothesized that the CHC viscosity could play an important role in a successful interaction. So far, chemical interactions have only been characterized in detail for one insect species, the cowpea weevil *Callosobruchus maculatus*. As different components in a CHC profile possess different viscosities and melting points and the CHC profile is most likely a solid–liquid mixture (Menzel et al., 2019), we assume that CHC differences will affect the interaction with the cribellate thread, thus enhancing or reducing the capture ability, i.e. adhesion force, of the cribellate thread towards different insects. This would suggest that cribellate threads might act as an additional selection pressure on the insect prey's CHC composition.

To test this hypothesis, we investigated how the interaction of CHC layers and cribellate spider threads is affected by CHC composition and temperature, and how CHCs differ in their tendency to migrate (i.e. to be soaked) into threads. We analysed which CHC classes migrated into the well-studied cribellate threads of the feather-legged lace weaver, *Uloborus plumipes* (Araneae: Uloboridae), and analysed the composition of original CHC extracts compared with those that had migrated into threads at different temperatures. To cover a large range of CHCs and avoid the influence of insect surface structures, CHCs were extracted from four distantly related insects with largely different CHC composition (house crickets, cowpea weevils, fruit flies and European red wood ants) and then applied onto metal wires. Insects of the respective families were additionally shown to be potential prey insects of cribellate spiders, though not necessarily for our species *U. plumipes*, whose natural prey spectrum has not yet been identified (Ludwig et al., 2018; Tsai and Pekár, 2019; Kennedy et al., 2020). Finally, we measured the adhesion force between thread and CHC layer to understand the interdependency of cribellate threads and CHCs of insects.

MATERIALS AND METHODS

Study animals

Uloborus plumipes Lucas 1846 were raised under room temperature (~21°C) and Central European daily rhythm. Once a week they were fed with *Drosophila melanogaster*. Water was provided once or twice per month by sprinkling the web. Threads of such sprinkled webs were not used for further studies.

Freshly hatched *Acheta domesticus* (Linnaeus 1758) (ca. 5 mm) were bought and kept for 5 days at 20°C in the lab, before freezing them at −20°C. They were stored frozen until further use. Wild-type *Drosophila melanogaster* Meigen 1830 were provided by a neighbouring lab and handled the same way. *Callosobruchus maculatus* (Fabricius 1775) were raised on dried cowpeas at lab temperature (~21°C) and frozen as described for *A. domesticus*. Workers of *Formica polyctena* Förster 1850 were collected from the wild and frozen at −20°C until further use. Please note that variance in CHC amount can impact the migration speed of CHCs into the cribellate thread (Bott et al., 2017). We cannot exclude that such variation could also influence the adhesion forces. We minimized potential variation by using the same number of insects with as similar a size as possible for further analysis.

Experimental setup and experimental procedure for CHC–thread interaction

For each of the four insect species, we produced 12 CHC extracts. These were used to coat three wires each for the different temperature treatments. Per replicate, the CHC-coated wires were then exposed to five spider threads at 20°C or 28°C (and for *F. polyctena* and *C. maculatus*, at 12°C in addition). After 7 days we analysed the chemical composition of CHCs in the threads, as well as that of the original insect extract. Typically, spiders capture prey within a few seconds; thus, adhesion forces develop if only a few CHCs have migrated into the thread (as also reflected in our adhesion measurements, see below). However, to collect enough CHCs for analysis, the threads had to be completely soaked with CHCs, which took around 7 days.

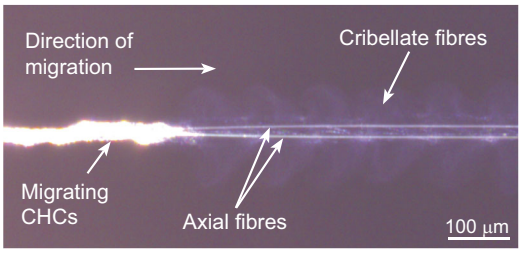

Fig. 1. Cuticular hydrocarbons (CHCs) of *Callosobruchus maculatus* migrating into the cribellate threads of *Uloborus plumipes*. Migration of CHCs into the thread is visible by a change in light reflection. Picture credit: Dominik Heidt.

For each CHC extract, 15 individual insects were covered with *n*-hexane for 10 min. After removing the insects, the hexane-containing CHCs were concentrated under a gentle nitrogen stream down to ca. 200 µl and transferred into a micro-inlet. We then coated metal paper clips with the CHC extracts. To this end, we placed one arm of a twisted clip into the CHC extract and let the solution evaporate. This procedure was performed twice per CHC sample to coat, first, two samples for the 20 and 28°C treatments, and then a third sample for the 12°C treatment (for *F. polyctena* and *C. maculatus*). Subsequently, five cribellate threads were picked from a web and transferred to the prepared paper clips, such that they spanned a line between the CHC-coated arm and a parallel, ca. 7 mm distant arm. This way, the CHCs could migrate (i.e. CHCs were soaked into the thread, visible by a change in reflection; Fig. 1) from the coated clip to into the threads. To analyse the native CHC extract, we re-dissolved the remaining CHCs in the micro-inlet in hexane.

We studied CHC migration at 20 and 28°C. For each temperature, we prepared 12 samples per insect species, and stored the threads on the clips in an incubator at the defined temperature for 1 week. For *F. polyctema* and *C. maculatus*, we performed an additional treatment at 12°C. After 7 days, the threads were removed from the paper clips by cutting both ends. The samples were transferred to a new GC-MS vial and covered with *n*-hexane. These extracts were then processed for chemical analysis as described below. We could not detect any pronounced migration in the samples of *D. melanogaster*, and the corresponding extracts did not contain any CHCs in identifiable quantities.

Chemical analysis

We injected 2 µl of concentrated extract into a gas chromatograph (GC 7890A, Agilent) coupled to a mass-selective detector (MSD 5975C, Agilent). The oven temperature started at 60°C for 2 min, then increased to 200°C at 60 K min^{-1}, and then further increased to 320°C at a rate of 4 K min^{-1}, where it was held constant for 10 min. MS data were obtained via electron ionization (70 eV). We used single ion monitoring (SIM) to reduce noise, and focused on the *m*/*z* peaks 55, 57, 69, 71, 83, 85, 97 and 99. Data were acquired with

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MSD ChemStation E.02.02 (Agilent). The peaks were integrated and aligned manually; identification was done based on retention indices and diagnostic ions. Samples of bad quality (peaks not clearly discernible as a result of low CHC abundance) were discarded. In total, we obtained chemical data for 9–12 replicates (median 12) per species (*F. polyctena*, *A. domesticus* and *C. maculatus*) and treatment (native CHC, threads treated at 20 or 28°C), and additionally for the 12°C treatment for *F. polyctena* (n=9) and *C. maculatus* (n=7). For *D. melanogaster*, none of the threads adsorbed CHCs in measurable quantities, leaving only the 12 native CHC extracts for this species.

Adhesion measurements

For the adhesion measurements, CHCs from 50 individuals per species were extracted as described above. They were placed in 1 ml vials and used to coat 5 paper clips. Single threads of *U. plumipes* were picked out of the web and transferred to a second twisted and uncoated paper clip. The CHC-coated paper clip was then affixed to a linear table and the *U. plumipes* thread sample to a microbalance (JB1603/C-FACT, Mettler Toledo AG). Both were connected to a computer that recorded position, velocity and negative weight (to calculate adhesion force). The coated paper clip was slowly moved downwards until contact with the thread was assured by measuring a change in weight. The adhesion force was measured as the maximum value obtained when retracting the sample from the thread at a speed of 1 mm s^{-1}. The measurements were repeated 15 times for each insect species, using a new thread and a new coated paper clip each time. As a negative control, the experiments were repeated with an uncoated hexane-washed paper clip as described above.

Scanning electron microscopy

Frozen specimens of all four insect species were taken and dried at room temperature for 1 day. Afterwards, they were placed onto conductive foil and covered with cribellate threads of *U. plumipes*. After 30 min, the samples were coated with gold (Hummer Technics Inc.) and the interaction between threads and insects was examined with a scanning electron microscope (SEM 525M, Philips AG).

Statistics

Chemical analyses were conducted for each insect species separately. We categorized CHCs into different classes: *n*-alkanes, trimethyl alkanes, alkenes, alkadienes and alkatrienes. Monomethyl and dimethyl alkanes were each divided into subcategories. This was to account for the result of Gibbs and Pomonis (1995) that, in monomethyl alkanes, the position of the methyl group influences the melting point: for a given chain length, melting points are highest for a 2-monomethyl alkane and decrease with increasing methyl group position up to 6, where they remain more or less constant. Therefore, we split monomethyl alkanes into 3-monomethyl alkanes, 4- and 2-monomethyl alkanes (these two CHCs cannot be separated chromatographically because of their similar retention times), 5-monomethyl alkanes and internally branched monomethyl alkanes (methyl group position 6 or higher). Similarly, we divided dimethyl alkanes into 'terminally branched' (first methyl group position <6) and 'internally branched' (first methyl group position ≥6). Then, we determined the relative abundance of CHCs in each of the above categories (such that they sum to 1) for each data point, and we performed a principal component analysis (PCA) of this dataset. The first two principal components were each analysed with a linear mixed-effects model. The principal component was the dependent variable; as fixed factors we used treatment (native CHCs versus CHCs from threads) and temperature (12°C/20°C/28°C; nested in treatment). Extract ID was included as random factor. The models were evaluated with a type II ANOVA (command *Anova*, package *car*). All analyses were done in R version 3.5.1 (http://www.R-project.org/). Adhesion forces were compared among insect species and negative control using ANOVA.

RESULTS

Visual characterization of the interaction

In a previous study, the interaction between cribellate threads and insects was mainly characterized visually by scanning electron microscopy (Bott et al., 2017). Testing our four insect species with the same method, we observed a very fast and obvious reaction between CHCs of *C. maculatus* and threads, visible as 'fused' nanofibres in the SEM (i.e. the nanofibres were embedded in CHCs; Fig. 2). The same has been reported previously. In addition, *A. domesticus* and *F. polyctena* showed an interaction with the thread, but neither as fast nor as pronounced as for *C. maculatus*. Only a very weak to almost no interaction was observed for *D. melanogaster* in the SEM.

Characterizing the CHC components interacting with the cribellate thread

The CHC composition of the cuticle of *A. domesticus* was dominated by alkadienes (unsaturated CHCs), whereas profiles of *C. maculatus* and *F. polyctena* were dominated by *n*-alkanes (saturated CHCs) and internally branched monomethyl alkanes and dimethylalkanes. The CHCs of *D. melanogaster*, in contrast, consisted mainly of saturated *n*-alkanes as well as 4- or 2-monomethyl alkanes and the rest were almost exclusively unsaturated alkenes (Fig. 3; Table S1). To analyse which of these CHC components interact with the cribellate thread, we measured the CHC composition in the threads after exposure to different temperatures.

A PCA on the hydrocarbon composition of *F. polyctena* yielded two principal component (PC) axes with eigenvalues >1 (Fig. 4). The first axis (PC1; eigenvalue: 5.73) explained 71.6% of the variance. PC1 values differed strongly between native CHC extracts and CHC extracts from threads (χ^2_1=863.1, P<0.0001). Native CHCs were characterized by more *n*-alkanes, whereas CHCs from threads possessed relatively more alkenes, and monomethyl (3-Me, 5-Me and internally branched), dimethyl and trimethyl alkanes. Among the thread extracts, experimental temperature affected hydrocarbon composition (χ^2_2=43.5, P<0.0001). In particular, threads treated at 28°C differed from those at the other two temperatures (both t>6, P<0.0001). Interestingly, thread CHCs of the 28°C treatment were most similar to native CHCs, while those from the 12°C treatment were most different (Fig. 4). The second PC axis (PC2; eigenvalue 1.13), explained 14.1% of the variance, but PC2 values did not differ between native CHCs and thread CHCs, nor among temperature treatments (both P≥0.1).

For *C. maculatus*, the PCA likewise yielded two eigenvalues >1, with PC1 (eigenvalue: 4.1) explaining 51.2% of the variation (Fig. 4). As in *F. polyctena*, PC1 values differed strongly between native and thread CHCs (χ^2_1=196.1, P<0.0001) as well as among temperature treatments (χ^2_2=16.5, P=0.00027). Again, thread CHCs in the 28°C treatment differed from those in the other two temperature treatments (both t≥3.0, P≤0.006) and were closest in composition to the native CHCs. Here, native CHCs were characterized by higher quantities of *n*-alkanes, 4-, 2- and 3-monomethyl alkanes, while thread CHCs had higher relative abundance of internally branched monomethyl alkanes and internally or terminally branched dimethyl alkanes.

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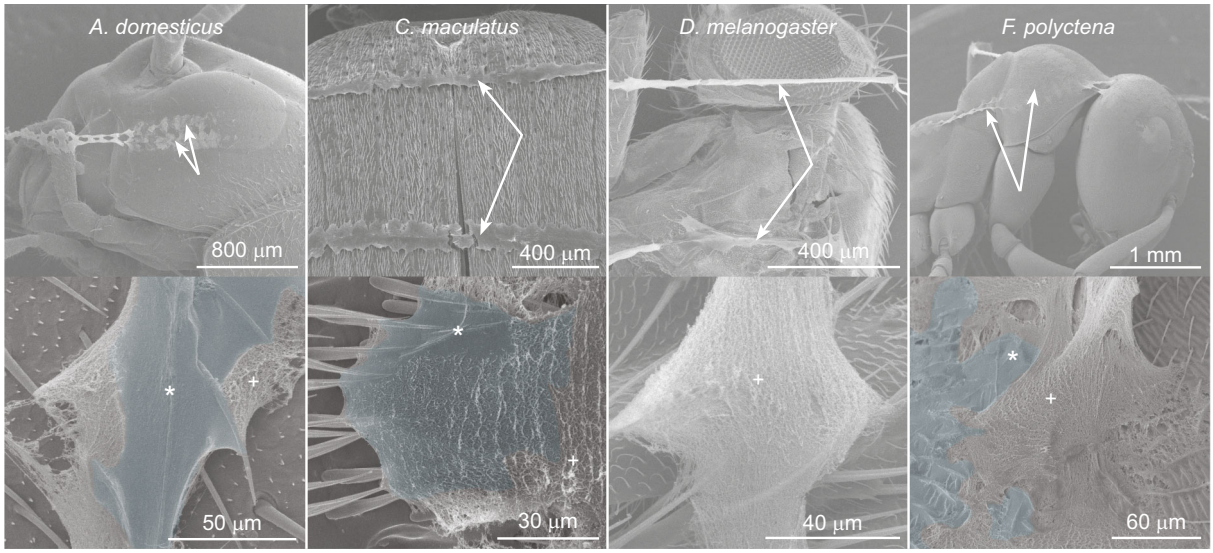

Fig. 2. Cribellate threads (arrows) in contact with four insect species. Scanning electron microscopy images show embedded areas (highlighted in blue) on *A. domesticus*, *C. maculatus* and *F. polyctena* (+, nanofibers; *, embedded nanofibres). Such areas were rarely visible in samples of *D. melanogaster*, where single nanofibres are easily distinguishable.

The proportions of 5-methyl alkanes and of trimethyl alkanes did not correlate with PC1. Values of PC2 (eigenvalue 1.8; 22.4% explained variance) did not differ between native CHCs and thread CHCs nor among temperatures.

Not surprisingly, similar results were found for CHCs of *A. domesticus* (Fig. 4). The PCA yielded two eigenvalues >1, with PC1 (eigenvalue: 4.87) explaining 54.1% of the variation and PC2 (eigenvalue: 1.56) explaining 17.3%. PC1 values differed strongly between native and thread CHCs (χ^2_1=140.2, P<0.0001) as well as among temperatures (χ^2_2=43.2, P<0.0001). Small but significant differences were also detected for PC2 values concerning the temperature treatment (χ^2_2=5.0, P=0.03), but not between native CHCs and thread CHCs. In contrast to the other species, unsaturated alkenes, such as alkatrienes and alkadienes, dominated the relative composition in thread CHCs, while native CHCs were characterized by higher relative quantities of *n*-alkanes, and various methylbranched alkanes.

Taken together, the relative amount of *n*-alkanes was always reduced in thread versus native CHCs, whereas the relative amount of internally branched dimethylalkanes, trimethylalkanes and unsaturated alkenes increased (Figs 4 and 5). Only in *A. domesticus* did we observed an increase of terminally branched alkanes in thread CHCs; this decreased in *F. polyctena* and *C. maculatus*. However, the CHC profile of *A. domesticus* contained different unsaturated hydrocarbons (alkenes, alkadienes, alkatrienes), whose relative amount was highly increased in the thread CHCs compared with native CHCs. For *D. melanogaster*, none of the threads adsorbed CHCs in measurable quantities. Hence, we were not able to quantify any differences for this species.

CHC composition influences adhesion of cribellate thread

Adhesion forces between cribellate threads and CHCs differed strongly among insect species (LM: F_3=8.29, P=0.00012; including negative control: F_4=11.8, P<0.0001; Fig. 6). This was mainly because adhesion of clips with *D. melanogaster* CHCs was significantly lower compared with that of all other species. At 19.7±28.3 µN, the adhesion force of *D. melanogaster* CHCs did not differ significantly from adhesion of cribellate threads to CHC-free surfaces (14.6±9.0 µN; see also Bott et al., 2017; Hawthorn and Opell, 2003). This reduced adhesion force matches our observation that a migration was detected neither by SEM nor in the GC-MS data. The highest force was measured on clips coated with *C. maculatus* CHCs (173.9±80.6 µN), followed by CHCs of *A. domesticus* (113.6±138.4 µN) and *F. polyctena* CHCs (105.2±52.0 µN). Similiar to these results, *C. maculatus* showed the strongest interaction in the SEM (Fig. 2).

DISCUSSION

Insect cuticular hydrocarbons attach to capture threads of cribellate spiders, most likely by means of capillary forces (Bott et al., 2017). Here, we show that the adhesion differs between insect species, and that different components of the CHC layer are adsorbed into the thread at different rates (Fig. 7). The differential behaviour of the

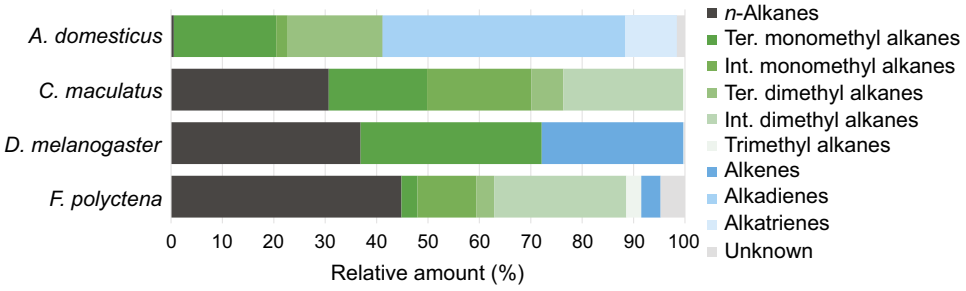

Fig. 3. CHC composition of the four insect species. The plots show the average proportion of the CHC classes in native extracts. Ter., terminally branched; Int., internally branched.

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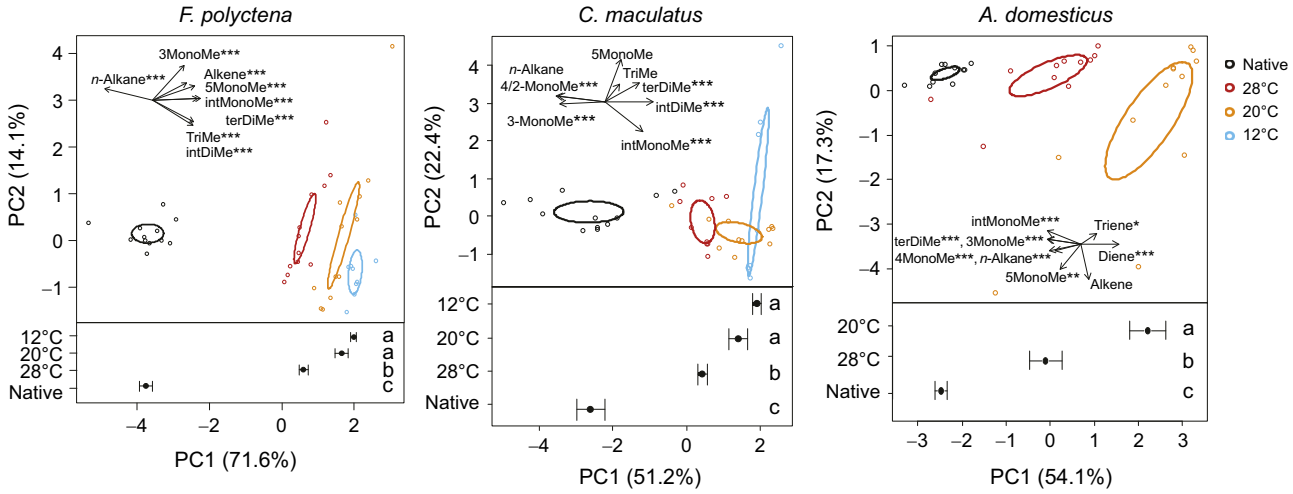

Fig. 4. Principal component analysis of the chemical composition of native CHCs and CHCs from threads, migrated at 28, 20 or 12°C. Composition was calculated as the relative abundance of the following substance classes: *n*-alkanes, 3-monomethyl alkanes (3MonoMe), 4- and 2-monomethyl alkanes (4/2MonoMe), 5-monomethyl alkanes (5MonoMe), internally branched monomethyl alkanes (intMonoMe), terminally branched dimethyl alkanes (1st methyl group at 3rd to 5th position, terDiMe), internally branched dimethyl alkanes (intDiMe), trimethyl alkanes (TriMe), alkenes, alkadienes (Diene) and alkatrienes (Triene). *n*-Alkanes and 4-and 2-methylalkanes overlap for *C. maculatus*. Below, PC1 values (means±s.e.m.) are shown. Asterisks indicate that the abundance of a substance class correlated significantly with PC1: ***P<0.0001, **P<0.01 and *P<0.05.

CHCs matches theoretical expectations based on viscosity and melting behaviour of CHC classes. Substances with high melting points (*n*-alkanes, followed by terminally branched monomethyl alkanes) were least likely to migrate into the threads, and hence threads that had been in contact with insect CHCs contained considerably lower amounts of these CHC classes compared with native extracts. At the other side of the gradient were alkenes and alkadienes, which, because of their low viscosity, occurred in high abundance in threads. This result yields two main conclusions: (1) the tendency to migrate into threads depends on physical properties, and can thus also be used to test biophysical hypotheses; (2) CHC composition influences the interaction and also the adhesion to cribellate threads. Aside from the composition, CHC quantity influences the migration speed into the thread (Bott et al., 2017). Concentration differences could also influence adhesion. However, in our opinion, concentration differences would not fully explain the observed differences in adhesion, especially the lack of adhesion in *Drosophila*. Hence, if cribellate spiders are abundant in a habitat, there should be a selection pressure on the prey species towards CHC blends with reduced adhesion.

Migration rates differ among CHCs

Insect *n*-alkanes have melting points of about 40°C and higher, with an average increase of 2 K per additional carbon atom. They pack tightly (as a result of van der Waals bonds), and thus are solid at ambient temperatures and can form crystals (Menzel et al., 2019; Maroncelli et al., 1982). Methyl-branched alkanes melt at a lower temperature, as the methyl branch hinders tight packing. Here, the branch position is critical: terminal methyl groups result in the highest melting points, because the remaining molecules can still aggregate tightly (Brooks et al., 2015), but the melting point decreases for more internally branched hydrocarbons (Gibbs and Pomonis, 1995). The same applies to compounds with more branches (e.g. dimethyl alkanes) or double bounds (e.g. alkenes), where the branches of the kink in the molecule at the double bound hinder tight packing. These theoretical differences in viscosity are reflected in the difference of

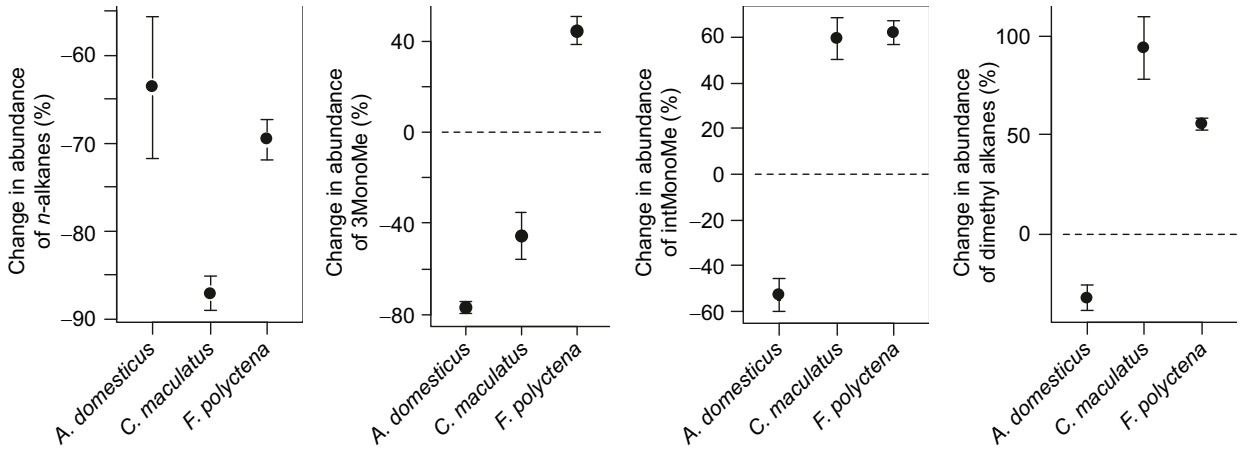

Fig. 5. Relative change in abundance of thread CHCs compared with native CHCs in three insect species. Data (means±s.e.m.) are shown for *n*-alkanes, 3-monomethyl alkanes (3MonoMe), internally branched monomethyl alkanes (intMonoMe) and dimethyl alkanes; data for the 20 and 28°C treatment were pooled. Note that *A. domesticus* does not possess internally branched dimethyl alkanes.

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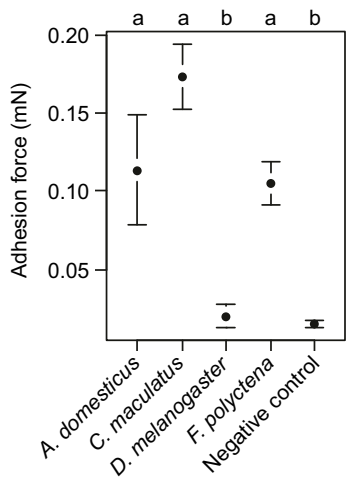

Fig. 6. Adhesion force measured between different CHC-coated metal clips and a cribellate thread. Data (means±s.e.m.) are shown for the indicated species and a negative control (a metal clip without any coating). Plots with the same letters are not significantly different according to Tukey's HSD based on a model including the four insect species and the negative control.

thread CHC composition versus native CHC extracts (Fig. 5). Especially at lower temperatures, thread extracts contained a far higher abundance of CHCs with lower melting points, and a far lower abundance of *n*-alkanes. Higher temperatures resulted in blends more and more similar to the native extracts, which is consistent with the hypothesis of more and more CHCs melting and/or decreasing in viscosity (viscosity decreases with temperature for all liquids). CHC layers of several ant species, for example, are completely liquid at temperatures above 40°C. Between 12 and 20°C, relatively few CHCs melt (Menzel et al., 2019). Hence, it is no surprise that hydrocarbons from the 28°C treatment were close to the native profiles, while those from the 20 and 12°C treatments were not significantly different from each other.

Whether a CHC class is more or less abundant in the thread compared with the native extract depends on the overall composition (Fig. 3). For example, compared with native CHCs, internally branched monomethyl alkanes were enriched in thread CHCs in *C. maculatus* and *F. polyctena*, but depleted in *A. domesticus* (Fig. 5). This is because roughly 50% of the profiles of *C. maculatus* and *F. polyctena* consist of CHCs that are more viscous or have higher melting points (*n*-alkanes and terminally branched monomethyl alkanes; Fig. 3), which is why internally branched monomethyl alkanes migrate relatively faster. In contrast, more than 50% of the *A. domesticus* profile consists of alkadienes and alkatrienes. Here, internally branched monomethyl alkanes migrate relatively slower and hence are depleted in thread CHCs (Fig. 5). For the same reason, 3-monomethyl alkanes, which have the highest melting point after *n*-alkanes, are enriched in thread CHCs of the *n*-alkane-rich *F. polyctena*, but depleted in thread CHCs of *C. maculatus* and *A. domesticus*, both of which possess more CHCs with lower viscosity or melting points. In this context, it seems surprising at first that, despite a high alkene content (>27%), *D. melanogaster* CHCs did not interact with the thread at all. This may be because the remaining CHC profile consists only of solid or highly viscous CHCs: our *Drosophila* profiles contained 36% *n*-alkanes and 35% terminally branched (4- and 2-) monomethyl alkanes (Fig. 3). Thus, CHC adhesion may have been low in *D. melanogaster* because most CHCs were too solid to migrate into the threads.

Melting points and viscosities of most insect hydrocarbons are still largely unknown, as they are expensive to synthesize artificially. Thus, for most substances, we have to rely on few data points, combined with theoretical considerations. However, our results nicely match theoretical predictions so far. Hence, this study also shows that cribellate threads provide a valuable tool to estimate the order in which different CHCs melt, and (potentially) test biophysical hypotheses on viscosity and phase behaviour.

Cribellate threads as a potential selection pressure on insects

In addition to corroborating current hypotheses about the physical properties of CHCs, this study also illuminates the interrelationship between insect prey and spider predators. Our results confirm that SEM images can give valuable first hints about the adhesion force between prey and capture thread (Bott et al., 2017). Of course, spiders react to prey in the web much faster than half an hour (the duration for SEM sample preparation) or even 7 days (the duration before CHC extraction), with typical reaction times of a few seconds (Eberhard, 1989; Blackledge and Zevenbergen, 2006). However, we observed that the interaction of the native insect cuticle in the SEM coincides with the measured adhesion force: neither pronounced interaction via the SEM nor enhanced adhesion force compared with wax-free control surfaces was verified for

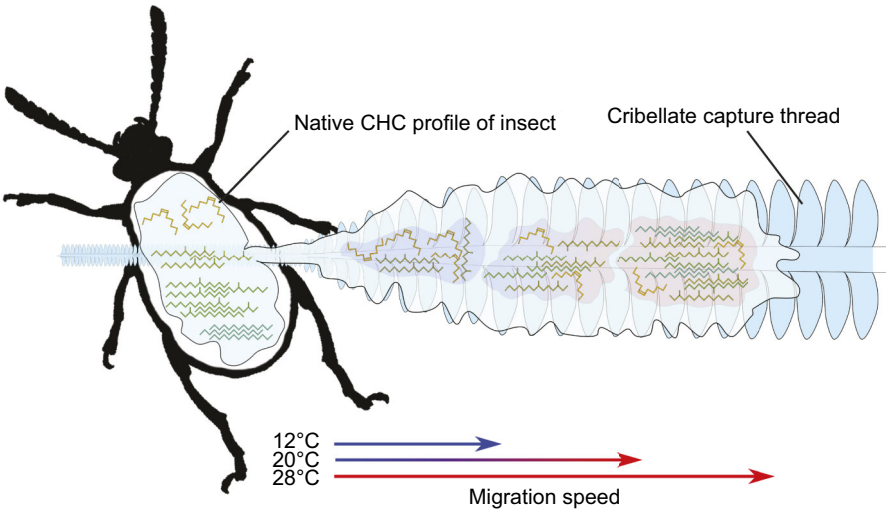

Fig. 7. Model of the migration of insect CHCs into a spider cribellate thread. With increasing temperature, the compositon resembles more and more the native profile of the insect. At low temperatures, CHCs (predominantly alkenes and dimethyl alkanes) migrate slowly. At higher temperatures, CHCs migrate faster, including also more viscous CHCs such as *n*-alkanes and monomethyl alkanes.

D. melanogaster, but all other insects showed interactions with cribellate threads, concomitant with increased adhesion force. Hence, we presume that CHC composition affects the adhesion force and thus the likelihood of prey being captured. This may represent a selection pressure by cribellate spiders on the insect CHCs that has not been recognized up to now. As CHC composition can evolve rapidly (Menzel et al., 2017b) and often differs drastically even between sister species (Morrison and Witte, 2011; Pokorny et al., 2014; Hartke et al., 2019), we do not know the CHC profiles of the ancient spiders' prey. Thus, we regard our study rather as a proof of principle that different CHC profiles experience different adhesion to cribellate threads. However, it will be difficult to show an evolutionary arms race between cribellate spiders and their prey's CHCs.

As mentioned above, the lack of interaction between *D. melanogaster* and cribellate threads is rather unexpected, because they are typical prey items and, in fact, the prey we feed to spiders in the lab. A recent publication showed a long retention of these flies in the capture threads of two cribellate species (Grannemann et al., 2019). Flying insects charge themselves electrically during flight and thus flying fruit flies attract cribellate threads (Joel and Baumgartner, 2017). Hence, other forces might drive the adhesion of fruit flies to cribellate threads. Adhesion force alone is, of course, only one aspect in a prey–predator interaction. Possibly, *D. melanogaster* is simply deficient in physical strength, unable to overcome even the smaller van der Waals forces acting between nanofibres and any surface. Lift forces of about 0.01 mN were measured for flying fruit flies, which would be too weak to escape even adhesion caused only by van der Waals forces (Yun et al., 2005). However, other potential prey can be much stronger: for ants, forces of 0.05 mN per leg were measured and for male lady beetles, even about 15 mN (traction force of complete animal) (Heepe et al., 2016; Reinhardt et al., 2009). Further studies should illuminate why species that are confirmed to be prey, like our fruit flies, are captured although their CHCs do not interact with the cribellate thread in this study.

Our study strongly suggests a benefit for cribellate spiders in hotter habitats: cribellate threads interact less with *n*-alkanes, but more with less viscous substances such as methyl-branched alkanes and especially unsaturated compounds (alkenes and alkadienes). This substance dependency, though, gets less pronounced at higher temperatures when CHCs are becoming less viscous, and we observed more native-like CHC profiles migrating into the thread. A previous study suggests a benefit for cribellate spiders in arid areas, as their capture threads have no glue which may dry out (Peters, 1987). Future studies should investigate the interrelationship between climate, prey captivity and CHC profile to further explore the evolutionary dynamics of this predator–prey system.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.-C.J., F.M.; Methodology: A.-C.J., F.M.; Validation: A.-C.J., F.M.; Formal analysis: D.S., F.M.; Investigation: A.-C.J., D.S., L.B., F.M.; Resources: A.-C.J., F.M.; Data curation: A.-C.J., D.S., L.B., F.M.; Writing - original draft: A.-C.J., F.M.; Visualization: A.-C.J., L.B., F.M.; Supervision: A.-C.J., F.M.; Project administration: A.-C.J., F.M.; Funding acquisition: A.-C.J., F.M.

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Data availability
Data are available from zenodo: https://doi.org/10.5281/zenodo.6256235.

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