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The specific expression patterns of sensory neuron membrane proteins are retained throughout the development of the desert locust *Schistocerca gregaria*

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ABSTRACT

The desert locust *Schistocerca gregaria* detects odorants through olfactory sensory neurons (OSNs) that are surrounded by non-neuronal support cells (SCs). OSNs and SCs are housed in cuticle structures, named sensilla found abundantly on the antenna in all developmental stages of the hemimetabolic insect. In insects, multiple proteins expressed by OSNs and SCs are indicated to play a pivotal role in the detection of odorants. This includes insect-specific members of the CD36 family of lipid receptors and transporters called sensory neuron membrane proteins (SNMPs). While the distribution pattern of the SNMP1 and SNMP2 subtypes in OSNs and SCs across different sensilla types has been elucidated for the adult *S. gregaria* antenna, their localization in cells and sensilla of different developmental stages is unclear. Here, we determined the SNMP1 and SNMP2 expression topography on the antenna of the first, third and fifth instar nymphs. Through FIHC experiments we found that in all developmental stages SNMP1 is expressed in OSNs and SCs of the trichoid and basiconic sensilla while SNMP2 is restricted to the SCs of the basiconic and coeloconic sensilla thus resembling the adult arrangement. Our results demonstrate that both SNMP types have defined cell- and sensilla-specific distribution patterns established already in the first instar nymphs and retained into the adult stage. This conserved expression topography underlines the importance of SNMP1 and SNMP2 in olfactory processes throughout the development of the desert locust.

Introduction

The ability to sensitively and precisely detect behaviorally relevant olfactory cues originating from conspecifics, food sources, or oviposition sites is of vital importance during all stages of insect life. This also applies to the hemimetabolic desert locust *Schistocerca gregaria* that can detect a plethora of relevant odorants including pheromones in all developmental stages (Ochieng and Hansson, 1999; Byers, 1991; Torto et al., 1996; Hassanali et al., 2005; Nakano et al., 2022; Njagi and Torto, 1996). Odor detection in the desert locust is accomplished via specialized olfactory sensory neurons (OSNs), mainly located on the antennae and palps (Greenwood and Chapman, 1984; Blaney, 1977; Lemke et al., 2020) where groups of OSNs are housed in a multitude of tiny hair-like structures called sensilla (Ochieng et al., 1998).

Olfactory sensilla comprise a perforated cuticle allowing for the entrance of odor molecules. Following sensillum entry, odor molecules are supposed to be transferred by odorant binding proteins (OBPs) in the sensillum lymph (Leal, 2013; Rihani et al., 2021) towards specific olfactory receptors in the membrane of the receptive dendrites of OSNs, that in insects mainly belong to the families of odorant receptors (ORs) and ionotropic receptors (IRs) (Fleischer et al., 2018; Wicher and Miazzi, 2021). At the base of the olfactory sensilla, OSNs are closely associated with non-neuronal support cells (SCs), which regulate the composition of the sensillum lymph and secrete OBPs into the fluid (Thurm and Küppers, 1980; Rihani et al., 2021). Thus, both the OSNs and the SCs of a sensillum express the proteins acting in the primary process of odor detection. In addition to OBPs and olfactory receptors, studies mainly in moths, flies and locusts (Rogers et al., 1997; Benton et al., 2007; Jiang et al., 2016; Cassau et al., 2022; Blankenburg et al., 2019) have demonstrated that certain OSNs and SCs also express subtypes of so-called sensory neuron membrane proteins (SNMPs) representing another group of proteins critical in the primary process of odor detection (Cassau and Krieger, 2021).

SNMPs are insect-specific members of the CD36 protein family (Rogers et al., 2001; Nichols and Vogt, 2008). Members of the CD36 protein family serve versatile functions including the reception and trans-

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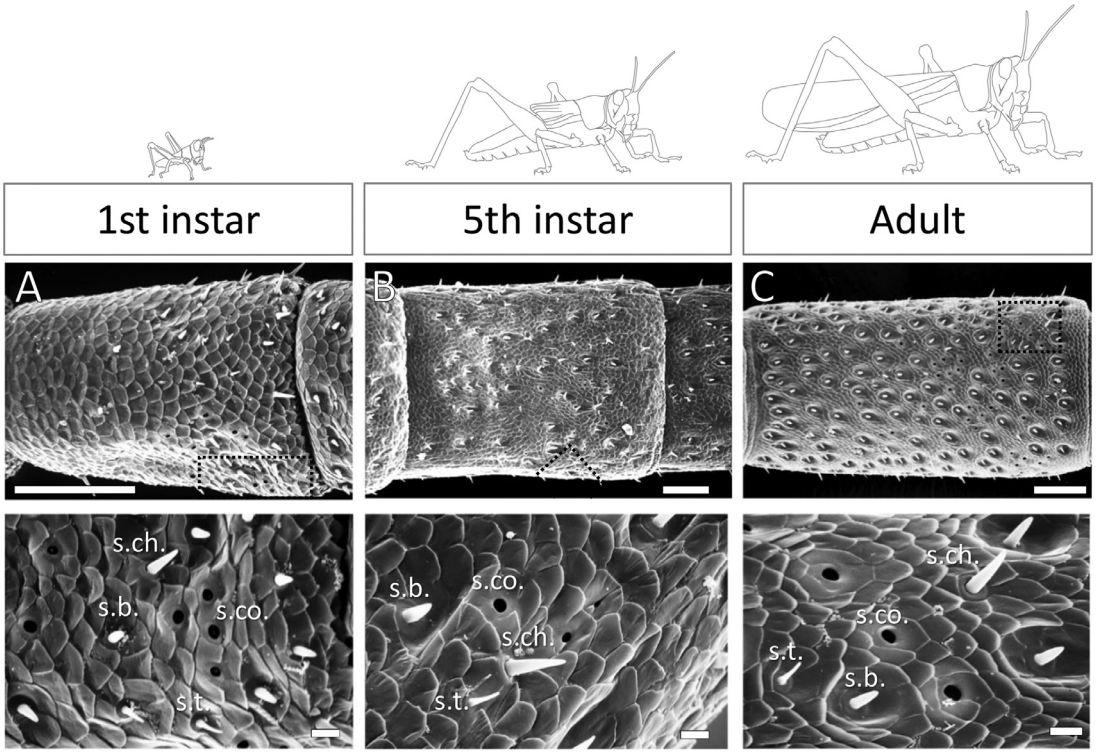

Fig. 1. Morphological classes of sensilla found on the antenna of *S. gregaria* in different developmental stages. The SEM images depict the middle antennal segments corresponding to: **A** first instar, segment 6, **B** fifth instar, segment 11 and **C** adult, segment 12. The lower row of images shows higher magnified areas of the regions boxed in the upper row. s.b. – sensillum basiconicum; s.ch. – sensillum chaeticum; s.co. – sensillum coeloconicum; s.t. – sensillum trichodeum. Scale bars: upper row = 100 μm; lower row = 10 μm.

port of lipids, lipophilic compounds and lipoproteins (Chen et al., 2022; Silverstein and Febbraio, 2009) and are distinguished by two transmembrane domains and a large ligand-interacting ectodomain (Gomez-Diaz et al., 2016; Pepino et al., 2014). Variable numbers of SNMPs have been identified across insect species and orders, forming a diverse SNMP gene family divided into four groups (SNMP1-SNMP4) based on phylogenetic relationships (Zhang et al., 2020; Zhao et al., 2020). Studies on the expression, localization and function of SNMPs in the olfactory system have concentrated on SNMP1 and SNMP2 subtypes found in all insect species studied so far (Cassau and Krieger, 2021). In *Drosophila melanogaster* (Benton et al., 2007; Jin et al., 2008; Gomez-Diaz et al., 2016) and heliothine moth species (Pregitzer et al., 2014; Liu et al., 2020), the SNMP1 subtype was found essential for the sensitive detection of lipophilic pheromone compounds by OSNs. Data from *Drosophila* suggest a function of SNMP1 as a co-receptor that might mediate a transfer of pheromones or other odorants from OBPs to cognate ORs via its tunnel-like ectodomain (Gomez-Diaz et al., 2016). Furthermore, a rapid activation and deactivation of pheromone-induced activity of OSNs was found to depend on SNMP1 (Li et al., 2014). While functional studies on the SNMP2-type are missing, the current data demonstrate broad expression in antennal SCs (Gu et al., 2013; Blankenburg et al., 2019; Jiang et al., 2016) and a localization in their microvilli membrane bordering the sensillum lymph (Cassau et al., 2022). Based on these finding and its homology to the CD36 protein family of lipid receptors and transporters, a role of SNMP2 in clearing the sensillum lymph from lipophilic odorants or their degradation products has been suggested (Cassau and Krieger, 2021; Forstner et al., 2008; Blankenburg et al., 2019).

Recently, we elucidated the expression topography of the SNMP1 and SNMP2 protein in the antenna of adult *S. gregaria* in the gregarious phase (Cassau et al., 2022). Using fluorescence immunohistochemistry (FIHC) and immunogold labelling experiments we revealed a detailed picture of their expression and localization across the OSNs and SCs of the three morphologically distinct olfactory sensilla types (Fig. 1). These are: broad and blunt sensilla basiconica, which house up to 30-50 OSNs, thin and sharp sensilla trichodea, bearing two to three OSNs, and sensilla coeloconica that are engulfed in pits in the antennal surface with three to four OSNs (Ochieng et al., 1998). Notably, sensilla chaetica are found as a fourth type of sensillum that are characterized by single pore openings at their tips and are supposed to serve gustatory and mechanosensory functions (Ochieng et al., 1998). In the adult desert locust, we found SNMP1 localized in a subset of OSNs innervating basiconic sensilla and in all OSNs innervating trichoid sensilla. Additionally, SNMP1 immune reactivity was detected in some SCs adjacent to the respective SNMP1-positive OSN clusters. In contrast, SNMP2 was localized solely in non-neuronal SCs of basiconic and coeloconic sensilla. Together our data suggest a role of SNMP2 limited to the functions of SCs, whereas SNMP1 seems to fulfill a dual function in the antenna of adult *S. gregaria*, i.e. in odor detection in OSN subpopulations as well as a function in certain SCs (Cassau et al., 2022).

Schistocerca gregaria is a hemimetabolic insect that develops through 5 nymphal stages into adulthood (Steedman, 1990). In contrast to the detailed picture revealed on the topography of SNMP1 and SNMP2 expression in adult desert locust antenna, the expression of the two SNMPs over the course of its development is unknown. So far, their developmental expression has been analyzed only by PCR on the transcript level demonstrating the presence of mRNA for SNMP1 and SNMP2 in the five nymphal instars of *S. gregaria* (Jiang et al., 2016). The *S. gregaria* antenna undergoes a successive development; with each moult, the locust antenna increase in length (from 3 mm in the 1st instar to 14 mm in the adults) as well as total number of segments (12 annuli in the 1st instar to 24 annuli in adults) (Ochieng et al., 1998; Chapman, 2002). This goes along with a massive increase in the total number of the olfactory sensilla types and consequently the overall number of OSNs on the antenna (Ochieng et al., 1998). Moreover, adult and juvenile stages

of locusts differ in their responsiveness to behaviorally relevant odorants (Obeng-Ofori et al., 1994; Torto et al., 1994), as well as in the expression levels of ORs, IRs and OBPs in in the olfactory appendages during development (Li et al., 2018; Wang et al., 2015; Xu et al., 2013). This implies differences in the expression of olfactory proteins across OSNs, SCs and sensilla between juvenile and adult locusts. Given this scenario, in this study we aimed at elucidating the SNMP1 and SNMP2 protein expression in different juvenile stages of *S. gregaria* in order to scrutinize whether the cell-type and sensilla-specific expression pattern of SNMPs observed in adult antenna is different in nymphal instars or alternatively already exists in the first nymphal stage and is retained during locust development. Towards this goal, we used SNMP-specific antibodies in immunohistochemical approaches to analyze the expression topography of the two SNMP types in cells of basiconic, trichoid and coeloconic sensilla on the antenna of first, third and fifth instar nymphs and compared it to adults.

Materials and methods

Animal rearing

Desert locusts, *Schistocerca gregaria*, were reared under crowded (gregarious) conditions as previously described (Seidelmann et al., 2000; Cassau et al., 2022) . Briefly, 100 to 150 individuals were kept in metal cages (50 × 50 × 50 cm) with metal grids at the bottom and at two sides. The photoperiod was 12L: 12D. The temperature was 34°C during the day and 27°C at night. The insects were fed with fresh wheat seedlings and flaked oats. The females were given cups of sand for oviposition, which were afterwards transferred to an empty cage for the development of the locusts. First, third and fifth instars were classified based on the number of eye stripes, as the number of stripes corresponds to their instar stage (Uvarov, 1966).

Scanning electron microscopy (SEM)

Antennae from cold anesthetized adults, fifth and first instar nymphs were carefully removed from the head and fixed for 15 min in a modified Carnoy's solution (ethanol, chloroform, acetic acid in a ratio of 3:1:1). For each stage one individual was examined. The samples were then dehydrated in a graded ethanol series of 60%, 70%, 80%, 90%, and 100% for at least 15 min in each solution, followed by 5 min in hexamethyldisilazane. The samples were placed onto a filter paper and left to dry overnight. Afterwards, they were mounted onto aluminum specimen stubs with double-sided adhesive pads. The following day, the samples were sputter-coated with gold for 200 s at 20 mA using a Balzers SCD 004 sputter coater (BAL-TEC, Balzers, Liechtenstein). The samples were examined with a Hitachi SEM S-2400 scanning electron microscope (Hitachi, Tokyo, Japan) at 12–18 kV and images were captured on ILFORD FP 4 black-and-white film (Harman Technology, Mobberley, UK). Contrast, brightness and tonal values of the images were adjusted to create uniformed figures.

Fluorescent immunohistochemistry (FIHC)

Fluorescence immunohistochemistry (FIHC) with the anti-SNMP-antibodies was performed as previously described (Cassau et al., 2022). For each stage we analysed at least 4 individuals. Third and fifth instars of *S. gregaria* were removed from the stock cultures and cold anesthetized on ice. The antennae were carefully dissected and immediately embedded into Tissue-Tek O.C.T. freezing medium (Sakura Finetek, Alphen aan den Rijn, the Netherlands) at -20°C. Cryosections (12 μM) of the samples were prepared with a Cryostar NX50 cryostat (Thermo Scientific) at −20°C. Sections were thaw-mounted onto SuperFrost Plus slides (Thermo Scientific) and kept cold at -20°C until encircling them using colourless ROTI®Liquid Barrier Marker (Carl Roth, Karlsruhe, Germany). Next, section were fixed by incubation of the slides for 20 min at 4°C with 4% paraformaldehyde (PFA) dissolved in phosphate buffered saline (PBS, 145 mM/L NaCl, 1.4 mM/L KH_2PO_4, 8 mM/L Na_2HPO_4, pH 7.4). Afterwards, the sections were rinsed at room temperature consecutively in PBS two times for 5 min, in PBS with 0.01% Tween20 for 5 min, in 50 mM NH_4Cl in PBS for 5 min and finally in PBS for 5 min. After incubating the samples in blocking solution (10% normal goat serum, 0.5% Triton-X100 in PBS) for 30 min at room temperature, the sections were incubated with the primary antibodies. The antibodies were diluted in blocking solution and added onto the sections, which were then incubated over night at 4°C in a humid box (rabbit-anti-SgreSNMP1-ab 1:500; rabbit-anti-SgreSNMP2-ab 1:100). The slides were then washed three times for 5 min with PBS and subsequently treated with goat-anti-rabbit AF488-conjugated secondary antibodies (1:1000) (Jackson ImmunoResearch, Ely, Great Britain), goat-anti-HRP Cy3 (1:400) (Jackson ImmunoResearch), and DAPI (1:500, Thermo Fisher Scientific) diluted in PBS, for 1 h at room temperature in a humid box. Finally, the slides were washed two times for 5 min in PBS, once for 5 min in dH_2O and then mounted in mowiol solution.

Whole mount immunohistochemistry

Preparation of histological sections from the first instar locust antenna for FIHC experiments were not suitable. We therefore attempted whole mount (WM) FIHC to obtain meaningful results. Finally, we found this method applicable for first instar antenna, which, due to their small size and relatively thin cuticle, allow for analysis of WM-preparation with the LSM.

For WM-FIHC experiments with first instar nymphs the same solutions were used as described for FIHC on sections. For this stage we investigated at least six individuals for SNMP1 and SNMP2, respectively. All incubation steps were conducted on an overhead shaker and in 0.3 ml reaction tubes. Antenna were carefully excised from the head and portioned into 2 mm sections then immediately transferred into 4% PFA in PBS with 0.5% Triton-x100 over night at 4°C. Afterwards, the samples were washed three times for 10 min with PBS followed by 10 min in 50 mM NH_4Cl in PBS and an overnight treatment in blocking solution at 4°C. The antennal fragments were then treated with the primary antibodies diluted in blocking solution (rabbit-anti-SgreSNMP1 1:200; rabbit-antSgreSNMP2 1:100) and incubated for 48h at 4°C. After washing the samples three times for 10 min with blocking solution, they were treated with goat-anti-rabbit AF488 (1:1000), goat-anti-HRP Cy3 (1:200) and DAPI (1:250) diluted in blocking solution for two days at 4°C. The antennal samples were then washed three times for 10 min with PBS and finally rinsed with dH_2O for at least 5 min and ultimately mounted with mowiol solution on microscope slides covered with cover slips.

Confocal microscopy

Sections from FIHC experiments and samples from the whole mount FIHC were analyzed on a confocal laser scanning microscope (LSM 880, Carl Zeiss Microscopy, Jena, Germany). Confocal image stacks of the fluorescence and transmitted-light channels were taken and used to generate either pictures representing single optical planes or projections of optical planes applying the ZEN software (Carl Zeiss Microscopy). Pictures were not altered except for adjusting the brightness or contrast for a uniform tone within a single figure.

Results

Sensilla types on the developing antenna

In order to confirm the conservation of the outer olfactory sensilla morphology during development, we first had a look if there are any obvious differences in the structures of the antennal sensilla types i.e. sensilla basiconica, s. trichodea, s. coeloconica and s. chaetica between

different developmental stages. Figure 1 shows sensilla on the approximate middle segments of a locust antenna from the first instar (segment 6), fifth instar (segment 11) and an adult (segment 12). In accordance with an earlier study (Ochieng et al., 1998), significant differences in the density of sensilla among these developmental stages of *S. gregaria* are immediately evident, with a dramatic increase in sensilla number from the first instar nymph to the adults. Yet, closer inspection of each individual sensillum type revealed no obvious differences between the olfactory sensilla of different developmental stages. Similar to adults, the sensilla basiconica of the first and fifth instars have wide shafts without a socket at their base and protrude out of flattened depressions in the cuticular surface. In all stages analyzed, the trichoid sensilla also appear similar; they are slightly arched and more slender compared to the basiconic sensilla. No obvious morphological changes during the development were also indicated for the coeloconic sensilla that are found in pits of the cuticular surface (Fig. 1C and Fig. S1). Higher magnification of a coeloconic sensillum reveals longitudinal ridges among the sensillum's shaft present in the first and adult stage (Fig. S1). Finally, the largest and massive gustatory/mechanosensory sensilla chaetica arise from basal sockets and look morphologically similar in the developmental stages analyzed (Fig. 1). Overall, although we examined only one individual per stage, the antennal sensilla types do not exhibit any notable morphological differences in locusts of different ages.

SNMP1 and SNMP2 expression in the antenna of the developing locust

In order to compare the distribution patterns of SNMP1 and SNMP2 proteins in the antenna of different juvenile stages and adults of the desert locust, we conducted comprehensive FIHC experiments. Towards this goal, we employed specific antibodies to discern the SNMP1 and SNMP2 expression topography together with an anti-HRP antibody and DAPI, that label neurons and nuclei, respectively.

First, we analyzed the fifth instar nymph, since their antennal morphology mostly resembles that of the adult locust. A representative result for the anti-SNMP1 immuno-labelling pattern obtained with an antennal section of the fifth nymphal stage is shown in Fig. 2A depicting several anti-SNMP1-ab immuno-labelled cells distributed across the section. Higher magnification of the boxed area in Fig. 2A shows two SNMP1-positive cells localized within a cluster of neuronal cells (Fig. 2B and C).

Next, we set out to determine the expression of SNMP1 in cells of the three different olfactory sensilla types of the fifth instar antenna (Fig. 2, D-I). In accordance with the finding that basiconic sensilla house about 30-50 OSNs (Ochieng et al., 1998), evaluation of this sensillum type revealed labeling of clusters of neurons with the anti-HRP antibody (Fig. 2D and E). The simultaneous visualization of neurons (anti-HRP) and SNMP1-positive cells (anti-SNMP1-ab) demonstrated that SNMP1 is expressed in a subpopulation of basiconic OSNs (Fig. 2, D-F, asterisks). In addition, anti-SNMP1-immune reactivity was also detected above the OSN cluster, labelling a large non-neuronal SC (Fig. 2, D-F, encircled area). For trichoid sensilla we found strong SNMP1 immunolabelling of multiple cells underneath the sensillum base (Fig. 2G and H). Merging the signals obtained with anti-SNMP1-ab and anti-HRP demonstrate expression of SNMP1 in an OSN (asterisk) that projects into the sensillum (Fig. 2G). The anti-SNMP1-ab clearly visualizes three more cells, which are non-neuronal and are situated between the SNMP1-positive OSN and the trichoid sensillum's base (encircled region Fig. 2, G-I). Thus, these FIHC results suggest that the trichoid sensilla of the fifth instar possess SNMP1-positive OSNs and multiple SNMP1-positive support cells. In contrast to basiconic and trichoid sensilla, inspection of the third olfactory sensillum type, i.e. coeloconic sensilla, did not reveal any SNMP1-positive cells.

Having established the SNMP1 distribution pattern in the fifth instar, we next set out to determine the expression topography of SNMP2 in this developmental stage (Fig. 3). Utilizing the anti-SgreSNMP2-ab we localized the SNMP2 protein in cells underneath basiconic and coeloconic sensilla (Fig. 3A). The magnified image of the basiconic sensillum from Fig. 3A depicts a large SNMP2-positive cell underneath the sensillum's base (Fig. 3, B-D). Through simultaneous visualization of the neurons by anti-HRP, it is evident that SNMP2 is not located in the OSNs of basiconic sensilla but most likely expressed in associated non-neuronal SCs (Fig. 3C and D, encircled). Similarly, for coeloconic sensilla we found strong anti-SNMP2-ab immune reactivity with non-neuronal cells just underneath the cuticle (Fig. 3 E-F). These SNMP2

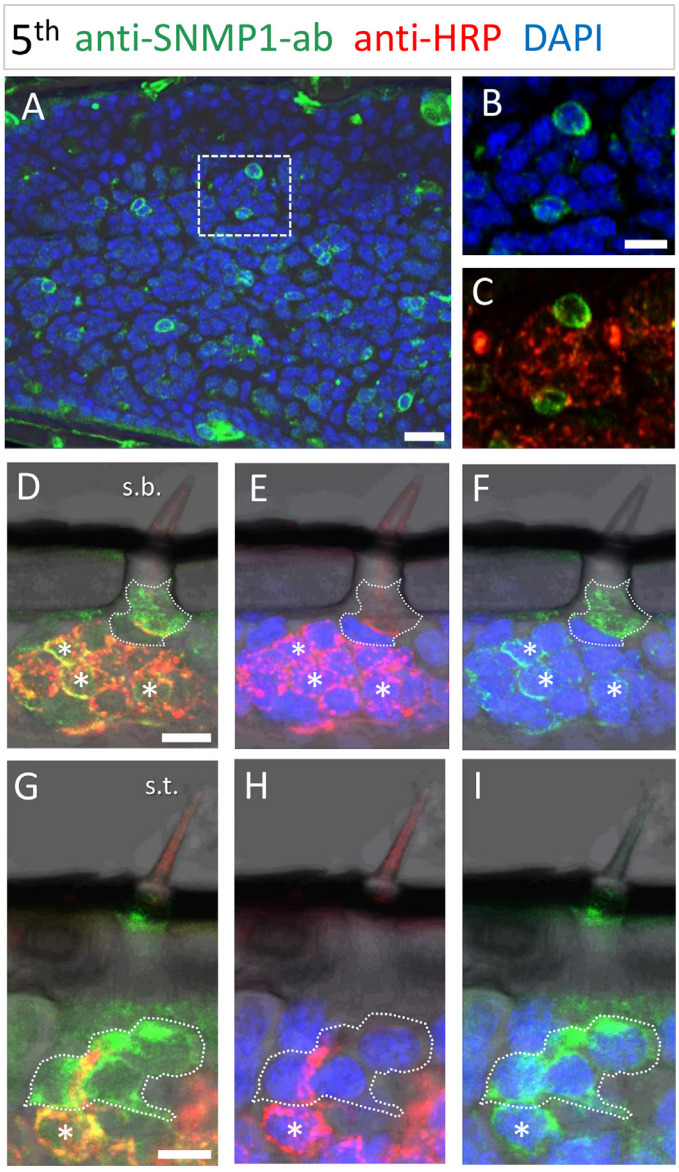

Fig. 2. SNMP1 expression in the antenna of the fifth instar of *Schistocerca gregaria*. SNMP1-positive cells were visualized by FIHC in longitudinal sections using anti-SgreSNMP1-ab (green). Neurons were identified by an anti-HRP-antibody (red) and nuclei were stained with DAPI (blue). **A** distribution of multiple SNMP1 cells in an antennal segment. **B, C** Higher magnification of the area boxed in (A) showing expression of SNMP1 (green) in a fraction of the OSNs (red). **D-G** SNMP1 is expressed in a subset of OSNs and in a support cell of a basiconic sensillum. **G-I** SNMP1-positive cells are localized underneath a trichoid sensillum. A, B, F, I show overlays of the green and blue fluorescence channels, while C, D, G represent overlays of the red and green channels. E and F, overlays of the red and blue channels. The transmitted light channel was overlaid with the fluorescent channels in A and D-I. Asterisks (*) denote SNMP1-positive OSNs. Encircled areas denote SNMP1-positive support cells. s.b. - sensillum basiconicum; s.t. - sensillum trichodeum. Scale bars: A = 20 μm; B, D, G = 10 μm.

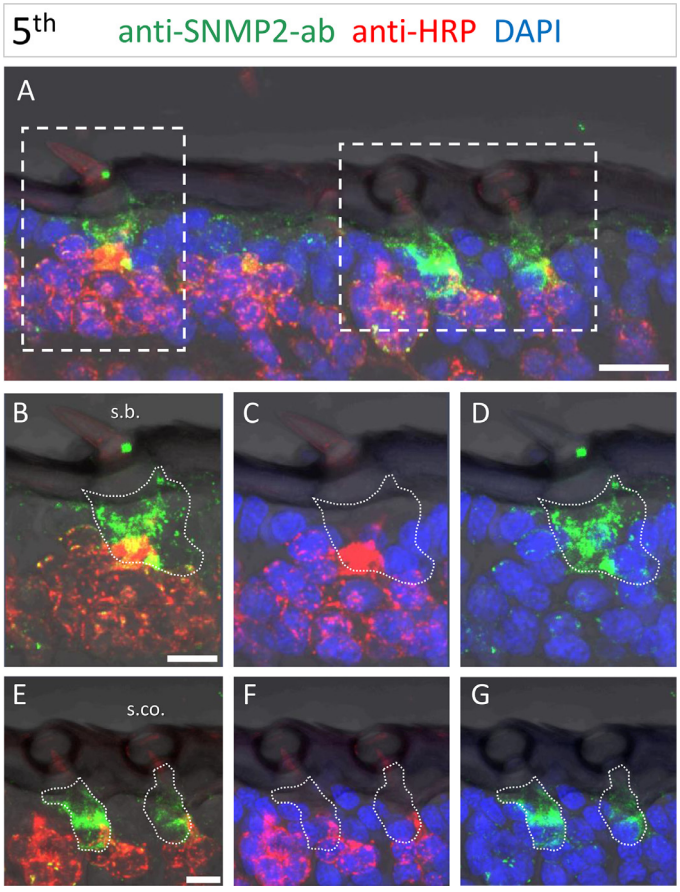

Fig. 3. Sensilla-specific distribution of SNMP2 in the 5th instar's antenna. SNMP2-positive cells were visualized by FIHC in the longitudinal section using anti-SgreSNMP2-ab (green). Neurons were visualized by an anti-HRP-antibody (red) and nuclei by staining with DAPI (blue). In all images the fluorescence channels were overlaid with the transmitted light channel. **A** Topography of SNMP2-expressing cells underneath sensilla shown in all fluorescence channels. **B-D** displays the area with the basiconic sensillum (s.b.) boxed in (A). Accordingly, **E-G** shows two sensilla coeloconica (s.co.) boxed in the right area in (A). B and C, overlays of the green and red fluorescence channels. C and F, red and blue channels. D and G, green and blue channels. The encircled areas denote SNMP2 positive support cells. Scale bars: A = 20 μm; B and E = 10 μm.

positive cells directly border a cluster of 3-4 OSNs that innervate the coeloconic sensilla (Fig. 3F and G, encircled). Along the antennal tissue, the anti-SgreSNMP2-ab did not generate any specific signals in neuronal cells.

Taken together, the analysis of the fifth instar antenna revealed that SNMP1 is expressed in OSNs of basiconic as well as trichoid sensilla and in addition in some SCs cells of these sensillum types. In contrast, SNMP2 was restricted to SCs of basiconic and coeloconic sensilla. Overall, these expression patterns match the SNMP1 and SNMP2 distribution patterns we previously obtained for the adult antenna (Cassau et al., 2022).

Approaching the SNMP1 and SNMP2 expression topography in the third instar nymph of desert locusts led to results similar to fifth instar nymphs. As demonstrated by Fig. S2, we found SNMP1 expression in a subset of OSNs of basiconic sensilla and in SCs of this sensillum type. In addition, all three OSNs of the trichoid sensillum were SNMP1-positive (Fig. S2, asterisks) as well as several adjacent non-neuronal SCs (Fig. S2, encircled area). In the antenna of the third instar nymph, anti-SNMP2-ab generated immune reactivity exclusively in non-neuronal cells of basiconic and coeloconic sensilla, suggesting that also in this developmental stage, the SNMP2 protein is restricted to SCs (Fig. S3).

Having revealed conserved antennal expression patterns for SNMP1 and SNMP2 in third and fifth instar nymphs, we set out to examine the antenna of the freshly hatched first instar nymphs. Because FIHC on sections was challenging and did not reveal clear results, we established whole mount FIHC (WM-FIHC) for the antenna of the first instar. In this developmental stage, the sensilla are most abundant in the distal half of the antenna (Fig. S4) covering both the ventral as well as the dorsal sides of the segments. With this background, we used segments one to six (Fig S4, A and B) in our analyses of the SNMP expression topography.

A representative result of a WM-FIHC using anti-HRP and anti-SNMP1-ab on the antenna of first instar nymph is depicted in Fig 4. Using the anti-HRP, we were able to visualize numerous antennal neurons (OSNs) organized in clusters in the whole mount preparation (Fig. 4A). In comparison, the SNMP1 antibody labeled a lower number of cells across an antennal segment (Fig. 4B). In the overlay of the anti-SNMP1 (green) and neuronal (red) labeling (Fig. 4C), it is immediately clear that SNMP1 is expressed in a subpopulation of OSNs also in this developmental stage. Closer inspection of a basiconic sensillum reveals that it is innervated by a large number of OSNs (Fig. 4D), comparable to basiconic sensilla of third and fifth instar nymphs (Fig. S2, Fig. 2). Of these OSNs, only some are SNMP1-positive (Fig. 4D and E, asterisks). Furthermore, similar to the later developmental stages, anti-SNMP1 immune reactivity was detected above the OSNs beneath the base of the sensillum's shaft, suggesting that SNMP1 is also expressed in the non-neuronal support cells in the first instar (Fig. 4D, encircled area). Upon inspection of the labeling of cells in first instar trichoid sensilla we found numerous SNMP1-positive cells (Fig. 4E and F), three of which were OSNs innervating the sensillum as indicated by positive anti-HRP labeling (Fig. 4G and H, asterisks). Moreover, SNMP1 immune reactivity was localized to three non-neuronal cells that are most likely SCs associated with the adjacent OSN cluster (Fig. 4G and H, encircled area). Similar to the other nymphal stages studied, no SNMP1 immune reactivity was detected in cells underneath coeloconic sensilla.

As with SNMP1, we assessed the SNMP2 distribution in the first instar's antenna utilizing anti-SgreSNMP2-ab in WM-FIHC experiments (Fig. 5). As shown by the representative image of an antennal segment, we found strong labelling of several cells indicative for SNMP2 expression (Fig. 5A). Through simultaneous anti-HRP labelling, we could show that SNMP2 that is not expressed in neurons i.e. OSNs but in the closely associated non-neuronal SCs (Fig. 5B). To define the topographic localization of SNMP2 in the antenna further, we assessed basiconic sensilla and detected SNMP2-positive cells in up to two cells above the cluster of OSNs, which project into the sensillum's shaft (Fig. 5, C-E, encircled). Similarly, SNMP2 immune reactivity was discovered beneath the non-neuronal SCs of coeloconic sensilla (Fig. 5, F-H, encircled areas). These SNMP2-positive cells are closely associated with the bordering OSNs, apparently enveloping the sensory neurons. Overall, the results obtained by analyzing the developing antenna strongly suggest that the specific expression topography of both SNMP1 and SNMP2 is already predetermined in the first instar nymph and subsequently retained throughout all stages of development towards the adult stage.

Discussion

In this study, we assessed the cell type- and sensilla-specific distribution pattern of the SNMP1 and SNMP2 proteins in the antennae of different developmental stages of the hemimetabolic desert locust *S. gregaria*. Initially, we inspected the outer morphology of the sensilla on the antenna of adults and the first and fifth juvenile stage. In agreement and extension of previous studies in *S. gregaria* (Ochieng et al., 1998) we identified four types of sensilla in each developmental stage i.e. the olfactory basiconic, trichoid and coeloconic sensilla and the presumed gustatory/mechanosensory sensilla chaetica, which all showed no obvious morphological differences along the development. Four basic sensillum types displaying similar morphologies were also reported for nymphal stages and adults of the related locust species *Schisto-*

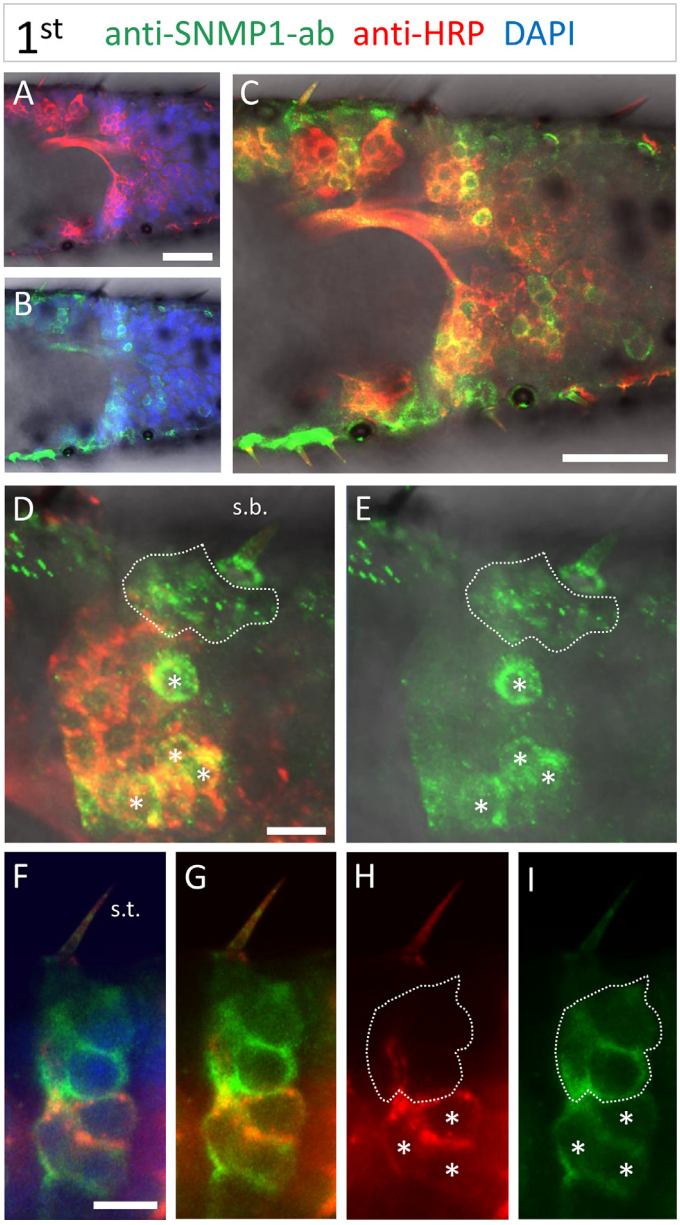

Fig. 4. SNMP1 expression in cells and sensilla of the antenna of first instar *S. gregaria.* Antennal segments were treated in whole-mount FIHC experiments using anti-SNMP1-ab (green), anti-HRP to visualize neurons (red) and DAPI to visualize nuclei (blue). **A-C** depiction of multiple OSN clusters and SNMP1-positive cells within an antennal segment, shown by different fluorescence channel combinations. **D-E** displays SNMP1-expression in subsets of OSNs and a support cell underneath a basiconic sensillum (s.b.). **F-I** multiple SNMP1-positive OSNs and support cells in a trichoid sensillum. A, red and blue fluorescence channels. B, green and blue channels. C, D, G red and green channels. E and I, green channel. F, green, blue and red channels. H, red channel. In A-E, the fluorescence channels are overlaid with the transmitted light channel. Asterisks (*) indicate SNMP1-positive OSNs. Encircled regions indicate SNMP1-positive support cells. Scale bars: A and C = 50 μm; D and F = 10 μm.

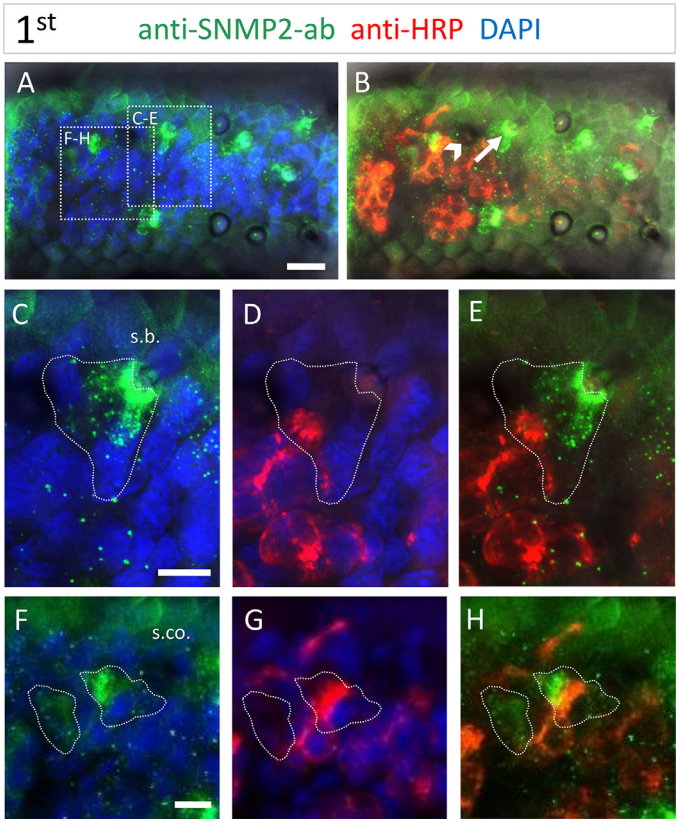

Fig. 5. Expression topography of SNMP2 in the antenna of the first instar of *S. gregaria.* The anti-SNMP2-ab was used in whole-mount FIHC experiments with antennal segments utilized to visualize SNMP2-positive cells (green). Anti-HRP and DAPI were used to visualize neurons (red) and nuclei (blue) respectively. **A-B** display the SNMP2-positive cells alone (A) or in combination with labelled neurons (B) indicating the non-neuronal expression of SNMP2 across the antennal segment. **C-E** higher magnified images of the right box in figure (A) illustrating SNMP2 expression in support cells underneath a basiconic sensillum (s.b.). **F-H** shows higher magnification of the left box in (A), depicting SNMP2-positive support cells associated with a coeloconic sensillum (s.co.). A, C, F, overlay of the green and blue fluorescence channels. B, E, H, green and red channels. D and G, red and blue channels. Encircled regions indicate SNMP1 positive support cells. In B the arrowhead denotes position of the coeloconic sensillum while the arrow indicates the position of the basiconic sensillum. Scale bars: A = 20 μm; C and F = 10 μm.

cerca americana (Chapman, 2002) and *Locusta migratoria* (Chapman and Greenwood, 1986) indicating a similar sensillum type equipment of the antenna in different locust species and their successive development stages.

Our FIHC analyses of the SNMP1 protein expression in the antenna of first, third and fifth nymphal instars revealed similar distribution patterns. We found the protein in all OSNs of trichoid and a subpopulation of OSNs in basiconic sensilla, but not in OSNs of coeloconic or chaetic sensilla. In addition, in all stages tested, SNMP1 was detected in non-neuronal SCs of trichoid and basiconic sensilla. The anti-SNMP1-ab labelling of SCs was most prominent for trichoid sensilla, enabling the detection of three SCs of this sensillum type in the first, third and fifth instar nymphs. Overall, the determined expression pattern of SNMP1 across cells and sensilla of antenna from nymphal instars perfectly matches the SNMP1 topography that we determined recently for the antenna of adult desert locusts (Cassau et al., 2022). This suggests similar functions of SNMP1 in the olfactory system of adults and in the different developmental stages of *S. gregaria*.

While currently no functional data exist for the role of SNMP1 in locusts, studies performed in the adult stage of the holometabolous fly *Drosophila melanogaster* and some moth species demonstrated a requirement of SNMP1 for a sensitive detection of pheromones and distinct plant odorants by OSNs of trichoid sensilla (Benton et al., 2007; Liu et al., 2020; Ronderos et al., 2014). Additionally, results from *Drosophila* suggest that SNMP1 may act as a co-receptor mediating the transfer of pheromone molecules from OBPs to neighboring ORs in the membrane of OSNs (Gomez-Diaz et al., 2016).

Given the proposed co-receptor function of SNMP1 in trichoid OSNs of the fly and of moths and its presence in adults and the larval stages of these holometabolous insects (Zielonka et al., 2016; Forstner et al., 2008; Herboso et al., 2011), it is conceivable that in the hemimetabolic desert locust SNMP1 may also serve in pheromone detection by trichoid OSNs and through certain OSNs of basiconic sensilla in adults and juvenile stages. This notion would fit with our yet limited knowledge of pheromone-detecting OSNs in locusts. In *S. gregaria,* single sensillum recordings from antenna revealed trichoid and basiconic OSNs are responsive to the putative pheromone (E,Z)-2,6-nonadienal and the pheromone phenylacetonitril (PAN), respectively (Ochieng and Hansson, 1999; Seidelmann and Ferenz, 2002). Moreover, in the migratory locust *Locusta migratoria* the aggregation pheromones 4-vinylanisol is detected by distinct OR35-expressing OSNs of basiconic sensilla in all developmental stages (Guo et al., 2020). However, given the finding that in *S. gregaria* adults (Pregitzer et al., 2017; Pregitzer et al., 2019) and nymphal instars (this study) SNMP1 shows a rather wide expression in OSNs and, moreover, SNMP1 was found co-expressed with at least 33 of the 119 ORs in adults (Pregitzer et al., 2019), it is unlikely that its function is limited to a role in pheromone detection. Instead, it may possibly have versatile roles in the reception of other behaviorally relevant odorants, such as olfactory cues indicating appropriate food sources for both nymphs and adults. Noteworthy, in adults (Cassau et al., 2022) and all juvenile stages studied we found SNMP1 in only a subset of the OSNs of basiconic sensilla, corroborating the presence of SNMP1-dependent and SNMP1-independent odorant detection pathways in all developmental stages of the locust.

Similar to *Drosophila* (Benton et al., 2007), we also found SNMP1 expressed in non-neuronal cells of olfactory sensilla indicating a dual role of this SNMP-type in OSNs and SCs. In contrast, the FIHC results obtained for SNMP2 with the antenna of the first, third and fifth instars revealed anti-SNMP2 labelling only of non-neuronal SCs located underneath basiconic and coeloconic sensilla. This SNMP2 expression pattern resembles the situation in adult locusts (Cassau et al., 2022) and demonstrates that also for this SNMP-type a cell-type- and sensilla-specific expression pattern is retained during development.

The function of SNMP1 and SNMP2 in the support cells of olfactory sensilla is presently unclear. As members of the CD36 family of lipid transporters/receptors, SNMPs in SCs are generally discussed as membrane proteins involved in the removal of lipophilic "waste products" from the sensillum lymph, such as odorants following receptor activation or their degradation products (Cassau and Krieger, 2021). In this context, CD36 proteins have been reported to act as membrane transporters of lipophilic compounds including fatty acids (Giovannucci and Stephenson, 1999; Kiefer et al., 2002; Wang et al., 2007; Yang and O'Tousa, 2007). Alternatively, it is possible that SNMPs facilitates the uptake of lipophilic compounds by a caveolae-dependent endocytotic pathway, as recently demonstrated for the CD36-mediated uptake of fatty acids into adipocytes (Hao et al., 2020).

In support of this concept, we recently localized both SNMP1 and SNMP2 in the apical microvilli membranes of SCs bordering the sensillum lymph of basiconic sensilla through immunogold labelling experiments (Cassau et al., 2022). Notably, SNMP1-expressing SCs, are found exclusively in sensillum types that also house SNMP1-expressing OSNs (trichoid, basiconic). Thus, it is tempting to speculate that SNMP1-expressing SCs may be involved in the clearance of compounds detected via SNMP1-expressing OSNs, whereas SNMP2 serves the removal of waste resulting from non-SNMP1-mediated odor detection. While SCs of trichoid sensilla only expresses SNMP1, we found both SNMP1 and SNMP2 in SCs underneath basiconic sensilla. However, our FIHC experiments with nymphal stages and adults (Cassau et al., 2022) did not allow us to unequivocally clarify the expression of the two SNMP-types in separate cells or a possible co-expression in some SCs.

In conclusion, we have revealed cell type- and sensillum type-specific expression patterns for SNMP1 and SNMP2 that are already established in the first instar and are retained to the adult stage. This conservation underlines the important roles of the two SNMP types in olfactory processes throughout development. Thereby, SNMP1 is indicated to have a function in a subpopulation of OSNs and in support cells, whereas SNMP2 serve a function only in support cells.

Data availability statement

Data are shown in the main manuscript or in the Supplementary Material. In addition, data from independent FIHC experiments conducted on different individuals of the 1st, 3rd and 5th nymphal stages of *S. gregaria* have been compiled in an "Additional Data File".

Author Contributions

Sina Cassau: Conceptualization, Investigation, Methodology, Analysis and Interpretation of data, Visualization, Writing – original draft; Angelina Degen: Investigation, Methodology, Analysis of data; Stephanie Krüger: Investigation, Methodology; Jürgen Krieger: Conceptualization, analysis and interpretation of data, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cris.2023.100053.

References

Benton, R., Vannice, K.S., Vosshall, L.B., 2007. An essential role for a CD36-related receptor in pheromone detection in *Drosophila*. Nature 450, 289–293.

Blaney, W.M., 1977. The ultrastructure of an olfactory sensillum on the maxillary palps of *Locusta migratoria* (L.). Cell Tissue Res. 184, 397–409.

Blankenburg, S., Cassau, S., Krieger, J., 2019. The expression patterns of SNMP1 and SNMP2 underline distinct functions of two CD36-related proteins in the olfactory system of the tobacco budworm *Heliothis virescens*. Cell Tissue Res. 378, 485–497.

Byers, J.A., 1991. Pheromones and chemical ecology of locusts. Biol. Rev. 66, 347–378.

Cassau, S., Krieger, J., 2021. The role of SNMPs in insect olfaction. Cell Tissue Res. 383, 21–33.

Cassau, S., Sander, D., Karcher, T., Laue, M., Hause, G., Breer, H., Krieger, J., 2022. The sensilla-specific expression and subcellular localization of SNMP1 and SNMP2 reveal novel insights into their roles in the antenna of the desert locust *Schistocerca gregaria*. Insects 13.

Chapman, R.F., 2002. Development of phenotypic differences in sensillum populations on the antennae of a grasshopper, *Schistocerca americana*. J. Morphol. 254, 186–194.

Chapman, R.F., Greenwood, M., 1986. Changes in distribution and abundance of antennal sensilla during growth of *Locusta migratoria* L. (Orthoptera: Acrididae). Int. J. Morphol. and Embryol. 15, 83–96.

Chen, Y., Zhang, J., Cui, W., Silverstein, R.L., 2022. CD36, a signaling receptor and fatty acid transporter that regulates immune cell metabolism and fate. J. Exp. Med. 219.

Fleischer, J., Pregitzer, P., Breer, H., Krieger, J., 2018. Access to the odor world: olfactory receptors and their role for signal transduction in insects. Cell. Mol. Life Sci. 75, 485–508.

Forstner, M., Gohl, T., Gondesen, I., Raming, K., Breer, H., Krieger, J., 2008. Differential expression of SNMP-1 and SNMP-2 proteins in pheromone-sensitive hairs of moths. Chem. Senses 33, 291–299.

Giovannucci, D.R., Stephenson, R.S., 1999. Identification and distribution of dietary precursors of the Drosophila visual pigment chromophore: analysis of carotenoids in wild type and ninaD mutants by HPLC. Vision Research 39, 219–229.
Gomez-Diaz, C., Bargeton, B., Abuin, L., Bukar, N., Reina, J.H., Bartoi, T., Graf, M., Ong, H., Ulbrich, M.H., Masson, J.F., Benton, R., 2016. A CD36 ectodomain mediates insect pheromone detection via a putative tunnelling mechanism. Nat. Commun. 7, 11866.
Greenwood, M., Chapman, R.F., 1984. Differences in numbers of sensilla on the antennae of solitarious and gregarious *Locusta migratoria L.* (Orthoptera: Acrididae). Int. J. Morphol. and Embryol. 13, 295–301.
Gu, S.H., Yang, R.N., Guo, M.B., Wang, G.R., Wu, K.M., Guo, Y.Y., Zhou, J.J., Zhang, Y.J., 2013. Molecular identification and differential expression of sensory neuron membrane proteins in the antennae of the black cutworm moth *Agrotis ipsilon*. J. Insect Physiol. 59, 430–443.
Guo, X., Yu, Q., Chen, D., Wei, J., Yang, P., Yu, J., Wang, X., Kang, L., 2020. 4-Vinylanisole is an aggregation pheromone in locusts. Nature 584, 584–588.
Hao, J.W., Wang, J., Guo, H., Zhao, Y.Y., Sun, H.H., Li, Y.F., Lai, X.Y., Zhao, N., Wang, X., Xie, C., Hong, L., Huang, X., Wang, H.R., Li, C.B., Liang, B., Chen, S., Zhao, T.J., 2020. CD36 facilitates fatty acid uptake by dynamic palmitoylation-regulated endocytosis. Nat. Commun. 11, 4765.
Hassanali, A., Njagi, P.G., Bashir, M.O., 2005. Chemical ecology of locusts and related acridids. Annu. Rev. Entomol. 50, 223–245.
Herboso, L., Talamillo, A., Perez, C., Barrio, R., 2011. Expression of the scavenger receptor class B type I (SR-BI) family in *Drosophila melanogaster*. Int. J Dev. Biol. 55, 603–611.
Jiang, X., Pregitzer, P., Grosse-Wilde, E., Breer, H., Krieger, J., 2016. Identification and characterization of two "Sensory Neuron Membrane Proteins" (SNMPs) of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). J. Insect Sci. 16, 33.
Jin, X., Ha, T.S., Smith, D.P., 2008. SNMP is a signaling component required for pheromone sensitivity in *Drosophila*. Proc. Natl. Acad. Sci. U.S.A 105, 10996–11001.
Kiefer, C., Sumser, E., Wernet, M.F., Von Lintig, J., 2002. A class B scavenger receptor mediates the cellular uptake of carotenoids in *Drosophila*. Proc. Natl. Acad. Sci. U.S.A. 99, 10581–10586.
Leal, W.S., 2013. Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Annu. Rev. Entomol. 58, 373–391.
Lemke, R.S., Pregitzer, P., Eichhorn, A.S., Breer, H., Krieger, J., Fleischer, J., 2020. SNMP1 and odorant receptors are co-expressed in olfactory neurons of the labial and maxillary palps from the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae). Cell Tissue Res. 379, 275–289.
Li, H., Wang, P., Zhang, L., Xu, X., Cao, Z., Zhang, L., 2018. Expressions of olfactory proteins in locust olfactory organs and a palp odorant receptor involved in plant aldehydes detection. Front. Physiol. 9, 663.
Li, Z., Ni, J.D., Huang, J., Montell, C., 2014. Requirement for *Drosophila* SNMP1 for rapid activation and termination of pheromone-induced activity. PLoS Genet. 10, e1004600.
Liu, S., Chang, H., Liu, W., Cui, W., Liu, Y., Wang, Y., Ren, B., Wang, G., 2020. Essential role for SNMP1 in detection of sex pheromones in *Helicoverpa armigera*. Insect. Biochem. Mol. Biol. 127, 103485.
Nakano, M., Morgan-Richards, M., Trewick, S.A., Clavijo-Mccormick, A., 2022. Chemical ecology and olfaction in short-horned grasshoppers (Orthoptera: Acrididae). J. Chem. Ecol. 48, 121–140.
Nichols, Z., Vogt, R.G., 2008. The SNMP/CD36 gene family in Diptera, Hymenoptera and Coleoptera: *Drosophila melanogaster, D. pseudoobscura, Anopheles gambiae, Aedes aegypti, Apis mellifera*, and *Tribolium castaneum*. Insect Biochem. Mol. Biol. 38, 398–415.
Njagi, P.G.N., Torto, B., 1996. Responses of nymphs of desert locust, *Schistocerca gregaria* to volatiles of plants used as rearing diet. Chemoecology 7, 172–178.
Obeng-Ofori, D., Torto, B., Njagi, P.G., Hassanali, A., Amiani, H., 1994. Fecal volatiles as part of the aggregation pheromone complex of the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). J. Chem. Ecol. 20, 2077–2087.
Ochieng, S.A., Hallberg, E., Hansson, B.S., 1998. Fine structure and distribution of antennal sensilla of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). Cell Tissue Res. 291, 525–536.
Ochieng, S.A., Hansson, B.S., 1999. Responses of olfactory receptor neurones to behaviourally important odours in gregarious and solitarious desert locust, *Schistocerca gregaria*. Physiol. Entomol. 24, 28–36.
Pepino, M.Y., Kuda, O., Samovski, D., Abumrad, N.A., 2014. Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. Annu. Rev. Nutr. 34, 281–303.
Pregitzer, P., Greschista, M., Breer, H., Krieger, J., 2014. The sensory neurone membrane protein SNMP1 contributes to the sensitivity of a pheromone detection system. Insect Mol. Biol. 23, 733–742.
Pregitzer, P., Jiang, X., Grosse-Wilde, E., Breer, H., Krieger, J., Fleischer, J., 2017. In search for pheromone receptors: certain members of the odorant receptor family in the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) are co-expressed with SNMP1. Int. J. Biol. Sci. 13, 911–922.
Pregitzer, P., Jiang, X., Lemke, R.S., Krieger, J., Fleischer, J., Breer, H., 2019. A subset of odorant receptors from the desert locust *Schistocerca gregaria* is co-expressed with the sensory neuron membrane protein 1. Insects 10, 350.
Rihani, K., Ferveur, J.F., Briand, L., 2021. The 40-year mystery of insect odorant-binding proteins. Biomolecules 11.
Rogers, M.E., Krieger, J., Vogt, R.G., 2001. Antennal SNMPs (sensory neuron membrane proteins) of Lepidoptera define a unique family of invertebrate CD36-like proteins. J. Neurobiol. 49, 47–61.
Rogers, M.E., Sun, M., Lerner, M.R., Vogt, R.G., 1997. Snmp-1, a novel membrane protein of olfactory neurons of the silk moth *Antheraea polyphemus* with homology to the CD36 family of membrane proteins. J. Biol. Chem. 272, 14792–14799.
Ronderos, D.S., Lin, C.C., Potter, C.J., Smith, D.P., 2014. Farnesol-detecting olfactory neurons in *Drosophila*. J. Neurosci. 34, 3959–3968.
Seidelmann, K., Ferenz, H.J., 2002. Courtship inhibition pheromone in desert locusts, *Schistocerca gregaria*. J. Insect Physiol. 48, 991–996.
Seidelmann, K., Luber, K., Ferenz, H.J., 2000. Analysis of release and role of benzyl cyanide in male desert locusts, *Schistocerca gregaria*. J. Chem. Ecol. 26, 1897–1910.
Silverstein, R.L., Febbraio, M., 2009. CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. Sci. Signal 2, 1–8.
Steedman, A., 1990. Locust Handbook. Overseas Development Natural Resource Institute, Chatham.
Thurm, U., Küppers, J., 1980. Epithelial physiology of insect sensilla. In: Locke, M., Smith, D.S. (Eds.), Insect biology in the future. Academic Press, New York, pp. 735–763.
Torto, B., Njagi, P.G.N., Hassanali, A., Amiani, H., 1996. Aggregation pheromone system of nymphal gregarious desert locust, *Schistocerca gregaria* (Forskal). J. Chem. Ecol. 22, 2273–2281.
Torto, B., Obeng-Ofori, D., Njagi, P.G., Hassanali, A., Amiani, H., 1994. Aggregation pheromone system of adult gregarious desert locust *Schistocerca gregaria* (Forskal). J. Chem. Ecol. 20, 1749–1762.
Uvarov, B.P., 1966. Grasshoppers and Locusts: A Handbook of General Acridology. Cambridge University Press.
Wang, T., Jiao, Y., Montell, C., 2007. Dissection of the pathway required for generation of vitamin A and for *Drosophila* phototransduction. J. Cell Biol. 177, 305–316.
Wang, Z., Yang, P., Chen, D., Jiang, F., Li, Y., Wang, X., Kang, L., 2015. Identification and functional analysis of olfactory receptor family reveal unusual characteristics of the olfactory system in the migratory locust. Cell. Mol. Life Sci. 72, 4429–4443.
Wicher, D., Miazzi, F., 2021. Functional properties of insect olfactory receptors: ionotropic receptors and odorant receptors. Cell Tissue Res. 383, 7–19.
Xu, H., Guo, M., Yang, Y., You, Y., Zhang, L., 2013. Differential expression of two novel odorant receptors in the locust (*Locusta migratoria*). BMC Neurosci. 14, 50.
Yang, J., O'tousa, J.E., 2007. Cellular sites of *Drosophila* NinaB and NinaD activity in vitamin A metabolism. Mol. Cell. Neurosci. 35, 49–56.
Zhang, H.J., Xu, W., Chen, Q.M., Sun, L.N., Anderson, A., Xia, Q.Y., Papanicolaou, A., 2020. A phylogenomics approach to characterizing sensory neuron membrane proteins (SNMPs) in Lepidoptera. Insect. Biochem. Mol. Biol. 118, 103313.
Zhao, Y.J., Li, G.C., Zhu, J.Y., Liu, N.Y., 2020. Genome-based analysis reveals a novel SNMP group of the Coleoptera and chemosensory receptors in *Rhaphuma horsfieldi*. Genomics 112, 2713–2728.
Zielonka, M., Gehrke, P., Badeke, E., Sachse, S., Breer, H., Krieger, J., 2016. Larval sensilla of the moth *Heliothis virescens* respond to sex pheromone components. Insect Mol. Biol. 25, 666–678.