Antennal Structure of Male and Female *Aphidius rhopalosiphi* DeStefani-Peres (Hymenoptera: Braconidae): Description and Morphological Alterations After Cold Storage or Heat Exposure

DELPHINE BOURDAIS, PHILIPPE VERNON, LILIANE KRESPI, JO LE LANNIC, AND JOAN VAN BAAREN

1 Université catholique de Louvain, Unité d’Ecologie et de Biogéographie, Place Croix du Sud 4-5, bât. Carnoy, 1348 Louvain-la-Neuve, Belgium
2 UMR 6553 CNRS - EcoBio, Université de Rennes I, Equipe Impact des Changements Climatiques, Station Biologique, 35380 Paimpont, France
3 Équipe d’Écobiologie des Insectes Parasitoides, Université de Rennes I, Avenue du Général Leclerc, 35042 Rennes cedex, France
4 Centre de Microscopie Electronique à Balayage et Microanalyse, Université de Rennes I, Campus de Beaulieu, Avenue du Général Leclerc, 35042 Rennes cedex, France
5 UMR 6553 CNRS - EcoBio, Université de Rennes I, Equipe Impact des Changements Climatiques, Campus de Beaulieu, avenue du Général Leclerc, 35042 Rennes cedex, France

KEY WORDS thermal stresses; cold storage; heat exposure; aphid parasitoids; antennal sensilla

ABSTRACT Several species of the genus *Aphidius* are used in biological control programs against aphid pests throughout the world and their behavior and physiology are well studied. But despite knowing the importance of sensory organs in their behavior, their antennal structure has never been described. We describe here the types and distribution of antennal sensilla in *Aphidius rhopalosiphi*, a larval parasitoid of several aphid species and observe how this antennal structure is modified after cold storage or heat exposure. Six types of sensilla were found on both male and female antennae. Male and female antennae differed in the total number of antennomeres (16 in males, 14 in females) and in the number and distribution of three of the six types of sensilla. After cold storage or heat exposure, we observed the appearance of a small number of abnormal sensilla.


INTRODUCTION

Several wasp species of the genus *Aphidius* (Hymenoptera: Braconidae) are used for biological control and are key organisms in studies of host–parasitoid interactions and behavioral ecology (Langer and Hance, 2004; Legrand et al., 2004b). *Aphidius rhopalosiphi* DeStefani-Peres, distributed throughout the holarctic zone (also introduced in Chile a long time ago), is a solitary parasitoid of the main aphid pests of cereal crops including *Sitobion avenae* Fabricius, *Metopolophium dirhodum* Walker and *Rhopalosiphum padi* L. (Langer et al., 1997; Latteur, 1973). Mass production and cold storage of these species have been recently studied (Colinet et al., 2006; Legrand et al., 2004a; Levie et al., 2005).

Mass rearing of insects is necessary for inundative biological control. However, obtaining a large number of individuals for optimum release and host synchronization often requires the insect’s exposure to low temperature to reduce their rate of development. Cold storage is the simplest and most efficient method to keep insects alive for periods of several months (Hofsvang and Hagvar, 1977). Parasitoids could be cold stored as eggs, larvae, or mummies (Levie et al., 2005; van Baaren et al., 2005), which means that all types of storage currently used stages prior to the metamorphosis. Despite its traditional use in biological control, cold storage is known to have deleterious consequences on the insects, including mortality, decrease in fecundity, selection of individuals with poor reproductive success, or male sterility (de Bach, 1943; Hanna, 1935; Levie et al., 2005; Polgar, 1986). More recently, it has been shown that the behavior of surviving individuals can be affected, such as the inability to follow an attractive odor (Herard et al., 1988), a decrease in mobility (Langer, 1999; Langer et al., 2004; Tezze and Botto, 2004), an alteration of learning capacities, patch exploitation behavior and mating capacities (Amice et al., submitted; van Baaren et al., 2005, in press). In *A. rhopalosiphi*, cold stored males are less able to locate the odor of virgin females, and thus mate less than control individuals (Bourdais, unpublished data). Antennal sensilla are known to be important sensory structures, involved in host location and host discrimination behaviors of females (Bleeker et al., 2004; Hays and Vinson, 1971; Roux et al., 2005), and female recognition by males (Battaglia et al., 2002; Bin et al., 1999; van Baaren et al., 1999). As the altered behaviors in *A. rhopalosiphi* involved the detection of odors, it seems to be possible that the ability to locate an attractive odor is dependant on odor perception, i.e., on the antennal sensilla.

*Correspondence to: Delphine Bourdais, Université catholique de Louvain, Unité d’Ecologie et de Biogéographie, Place Croix du Sud 4-5, bât. Carnoy, 1348 Louvain-la-Neuve, Belgium. E-mail: bourdais@ecol.ucl.ac.be

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The consequences of an exposure to nonlethal low temperatures have been well-studied in parasitoids, while exposure to high sublethal temperatures has never been analyzed. Studies on the effects of low temperatures are relevant to cold storage for biological control. However, understanding the consequences of heat exposure is fundamental, because even without climate change, insects may be exposed to high temperature due to natural fluctuations. Global climate change is expected to increase frequencies and amplitudes of such events (IPCC, 2001).

In the present study, we first describe the antennal sensory structures of male and female A. rhopalosiphi using scanning electron microscopy. Indeed, only few studies have reported the sensilla of parasitoids (Olson and Andow, 1993) and no such studies are known in Aphidiinae. Secondly, we compare the sensilla of control individuals to males and females exposed to cold or heat thermal stresses. Our hypothesis is that the behavioral alterations observed in surviving individuals after thermal stress (Herard et al., 1988; Langer, 1999; van Baaren et al., 2005) are due to sensorial deficiency. One way to test this hypothesis is to verify if the morphology, numbers, and distributions of each type of sensilla could be modified by thermal stress during the pupal stage.

### MATERIALS AND METHODS

#### Plants and Insects

*Aphidius rhopalosiphi* were obtained from *Sitobion avenae* Fabricus (Homoptera: Aphididae) mummies collected in cereal crops around Rennes (Lat. 48° 06'10"; Long. –01° 47'39") (Brittany, France). This region has an oceanic climate: temperatures vary from 1 to 5°C in winter and from 18 to 20°C in summer. However, periods of chill and extreme heat are also quite common, with a mean of 20–30 days per year below 0°C and of more than 40 hot days above 25°C.

Aphids and parasitoids were reared in the laboratory on a mixed-age culture of *S. avenae* originating from one parthenogenetic female collected in 1990 in the same area (SA1 clone, INRA-Zoology Collection). Aphids were reared on winter wheat, *Triticum aestivum*, and had a carina shape and a length of 100 μm long, but females had a slender shape (Fig. 1a). Females had 14.12 ± 0.05 (n = 17) antennomeres, whereas males had 16 ± 0.05 (n = 68). Each antennomere was about 100 μm long, but all antennomeres were thinner in females (30 μm) than in males, for which all antennomeres had the same diameter (50 μm) (Figs. 1b and 1c).

#### Results

### Antennae

Antennae of males and females had a long, uniform slender shape (Fig. 1a). Females had 14.12 ± 0.05 (n = 17) antennomeres, whereas males had 16 ± 0.05 (n = 68). Each antennomere was about 100 μm long, but there was a sexual dimorphism in their diameter: the first three antennomeres were thinner in females (30 μm) than in males, for which all antennomeres had the same diameter (50 μm) (Figs. 1b and 1c).

### Sensilla Types

Six different types of sensilla were found on both male and female antennae, and their numbers on each antennomere are given in Table 1. Sensilla Placodea or Multiporous Plate Sensilla. Most of the antennomeres were covered by sensilla placodea with length equal to the total length of the antennomere. These sensilla were found on all male and female antennomeres except the first of females (Fig. 1b). There were 8–11 sensilla per antennomere in males and 0–11 in females (Table 1), distributed fairly regularly all around the flagellum. However, the last antennomere of females showed two successive rings of placodea (Fig. 1f), as if two different antennomeres were merged. This phenomenon was rarely observed in males (11%, n = 68), for which the last antennomere usually showed only one ring of placodea. Sensilla placodea were more numerous in males than in females (Table 1), and had a carina shape and a length of 100 μm. They

### Scanning Electron Microscopy

After being anesthetized by CO₂, entire, newly-emerged parasitoids were killed by immersion in a buffered solution of glutaraldehyde 2.5% (pH 7.2) for 48 h at 4°C. They were then placed in cacodylate buffer 0.1 M pH 7.2 and serially dehydrated in alcohol (60%, 70%, 80%, 90%, 95%, and 100%). Preparations were kept in 100% acetone until processed for microscopy. Before observations, samples were critical point dried using CO₂ with a Blazers CPD-010 (Blazers, Liechtenstein) and gold-palladium coated using a JEOL JFC-1100 sputter unit (JEOL, Tokyo, Japan). Observations were performed at 2.5 kV with a JEOL SEM-6400.

We used the sensilla denomination and classification of Bleeker et al. (2004), Zacharuk (1980, 1985), and Keil (1999). Two males and two females from the control population were observed (Table 1). A total of six males (1 for 7, 21, and 28 days and 3 for 14 days), and two females exposed to cold were observed. Two individuals of each sex exposed to heat were observed (Table 2).

### Number of Antennomeres

For each treatment, the general form of the antennae and the number of antennomeres per antennae were recorded under a binocular microscope. We observed 68 control males and at least 10 males for each cold treatment (at 4°C: 7 days, n = 51; 14 days, n = 17; 21 days, n = 19; 28 days, n = 10) and heat treatment (at 28°C: 2 days, n = 19 and 4 days, n = 30). Females were also observed in the same way (controls, n = 17; at 4°C: 28 days, n = 17; 42 days, n = 7; at 28°C: 4 days, n = 14; and at 30°C: 4 days, n = 6).

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TABLE 1. Number of different types of sensilla on each antennomere in males (M) and females (F) of *A. rhopalosiphi*, from the proximal (1) to the distal (14 for females, 16 for males) part of antennae (2 males and 2 females were observed)

<table>
<thead>
<tr>
<th>Antennomere</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. placodea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>137–144</td>
</tr>
<tr>
<td>M</td>
<td>5–8</td>
<td>7–8</td>
<td>8–9</td>
<td>9</td>
<td>7–9</td>
<td>8–11</td>
<td>9–10</td>
<td>9</td>
<td>9–10</td>
<td>8–10</td>
<td>9</td>
<td>8–10</td>
<td>9</td>
<td>8–10</td>
<td>9</td>
<td>8–10</td>
<td>9</td>
</tr>
</tbody>
</table>

Putative function

**S. placodea**
They have multiple pores and are known to have an olfactory function in different groups: honeybees (Akers and Getz, 1992), scarab beetle (Hanson et al., 1999), and in braconid parasitoids (Ochieng et al., 2000), where its structure has been completely described (Barlin and Vinson, 1981).

**S. trichodea**
These sensilla have no pore and are considered to be mechanoreceptors, due to their socket-like insertion into the antennal cuticle (Keil, 1999; Ochieng et al., 2000).

**S. trichodea**
Numerous on each antennomere

**S. trichodea**
They have probably an olfactory function due to their numerous pores on the whole surface, and their internal morphology.

**S. trichodea**
They are considered to be contact chemoreceptors and involved in gustatory function (Barbarossa et al., 1998).

**S. coeloconica**
Olson and Andow (1993) suggested an olfactory function for this sensillar type in *Trichogramma nubilale*, but as revealed in several insect species (Altner et al., 1983), it may also be involved in thermoreception or hygroreception.

**S. coeloconica**
Sensilla coeloconica type II could also be thermoreceptors or hygroreceptors (Altner and Prillinger, 1980).

The putative function of these sensilla can be deduced from the number of pores (Bleeker et al., 2004; Keil, 1999) and by electrophysiological studies.
were completely covered by pores of ~1 nm in diameter, distributed in different rings (Figs. 1c and 2g).

**Sensilla Trichodea Nonporous (NP).** These were the most abundant type on all antennomeres and were distributed in several rows between each sensilla placodea (Fig. 1c). We observed that the first antennomere of females had less sensilla than others, and sometimes none (Fig. 1b). They were inserted in a socket (corresponding to a slight elevation of the cuticle), had a droplet shape at the tip (Fig. 1b). Their length was about 30 µm and their diameter 1 µm at the base.

**Sensilla Trichodea With Wall Pores (WP).** These were present on all male antennomeres and were absent only on the first one in females. They were slightly more numerous in females and their distribution on each antennomere was different. In males, sensilla were on the proximal part of each antennomere, whereas in females, some were on the proximal part as in males and others were on the distal part. We observed usually one or two sensilla per antennomere in males, compared with one to five in females. These sensilla have a smooth cuticle, covered by several pores, and they have a socket. They were slightly bulbous at the base with a diameter of ~2 µm and a conical tip (Figs. 1c and 2a).

**Sensilla Trichodea With Tip Pore (TP).** These were present on all antennomeres in one ring for males (Fig. 1c) and two for females (one on the middle of the antennomere and one at the distal part). They were more numerous in females, mainly because of the second ring (Table 2). These sensilla have a classical conical shape with pores on the tip (Fig. 2b) with a length of ~20 µm and a diameter of ~1 µm at the base. They emerged from the other types of sensilla because they were orientated perpendicularly to the antennomere.

**Sensilla Coeloconica Type I.** These were as numerous in males as in females and were present on the distal part of antennomeres 10–15 (zero to one per antennomere). Bleeker et al. (2004) described them as a peg protruding from a pit in a donut-shaped ring. They had a maximal width of 0.8 µm and were 4 µm in length. The pit had an external diameter of 6 µm and the peg was deeply striated (Fig. 2c).

**Sensilla Coeloconica Type II.** There was ≤ of them on each of antennomeres 5–15 in both males and females. These sensilla had an oval dot shape of 4-µm-length and 3-µm width, with a small bulb of 0.6 µm diameter in the middle (Fig. 2e).

### Effects of Cold Storage and Heat Exposure

In males, the number of sensilla placodea and sensilla trichodea WP decreased in case of cold exposure, whereas in cold-stored females, the number of sensilla trichodea TP decreased (Table 2). The number of the two types of coeloconica was not altered by any kind of exposure in males or females (Table 2).

One of the most striking effects of cold or heat exposure was the appearance of a small number of abnormal sensilla (1–15 abnormal sensilla per antennomere). The two types of coeloconica were both damaged (Figs. 3d–3h). The sensilla coeloconica type I appeared to be mainly affected at the extremity but sometimes also at the base, whereas the type II seemed to be completely modified. For both males and females, we found sensilla trichodea WP with an abnormal tip (Figs. 3a and 3b for cold and heat exposure, respectively). The tips of the sensilla were bifid or trifid. Placodea sensilla seemed very sensitive to thermal stresses because abnormal sensilla (curved instead of straight, Fig. 3i) occurred in all exposed individuals after only 1 week of cold storage. When insects are exposed to cold for more than 3 weeks, each antennomere had one or more abnormal placodea.

These treatments induced modifications in the number of antennomeres in males but not in females. Exposed males had fewer antennomeres than control males (ANOVA, \( F = 4.41, df = 6, P = 0.00024 \)), mainly because of the presence of abnormal antennae extremities increased with longer distal antennomeres, as if two antennomeres had merged (Fig. 4).

### DISCUSSION

This article describes the antennal sensilla of the aphid parasitoid *A. rhopalosphi* and the effect of cold and heat exposures on the morphology of these antennal sense organs. Six different types of sensilla were found and were common in both sexes. These six types have been previously described in other Hymenoptera (e.g., Bleeker et al., 2004; Ochieng et al., 2000; Roux et al., 2005; van Baaren et al., 1996, 1999).

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**Table 2. Total number of different types of sensilla on the antennae in males (M) and females (F) of A. rhopalosphi, in function of cold or heat exposure**

<table>
<thead>
<tr>
<th>Sensilla type</th>
<th>Control</th>
<th>Cold exposure</th>
<th>Heat exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. placodea</td>
<td>137–144 (M)</td>
<td>91–131 (M)</td>
<td>118–156 (M)</td>
</tr>
<tr>
<td>S. trichodea WP</td>
<td>72–97 (F)</td>
<td>71–72 (F)</td>
<td>86–87 (F)</td>
</tr>
<tr>
<td>S. trichodea TP</td>
<td>20–25 (M)</td>
<td>10–17 (M)</td>
<td>19–27 (M)</td>
</tr>
<tr>
<td>S. coeloconica I</td>
<td>29–31 (F)</td>
<td>24–33 (F)</td>
<td>32–42 (F)</td>
</tr>
<tr>
<td>S. coeloconica II</td>
<td>66 (M)</td>
<td>56–70 (M)</td>
<td>67–68 (M)</td>
</tr>
<tr>
<td>S. coeloconica III</td>
<td>117–129 (F)</td>
<td>92–102 (F)</td>
<td>120–135 (F)</td>
</tr>
<tr>
<td>Types of abnormal</td>
<td>None</td>
<td>Placodea,</td>
<td>Placodea,</td>
</tr>
<tr>
<td>sensilla observed</td>
<td></td>
<td>trichodea WP,</td>
<td>trichodea WP,</td>
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<td></td>
<td></td>
<td>coeloconica type I,</td>
<td>coeloconica type I,</td>
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<tr>
<td></td>
<td></td>
<td>and coeloconica type II</td>
<td>and coeloconica type II</td>
</tr>
</tbody>
</table>

For cold exposure, a total of 6 males and 2 females were observed, and 2 individuals of each sex for heat exposure and control.
Comparison With Sensilla Types Found in Other Species

The putative functions of the described sensilla in this study are given in Table 1. Sensilla trichodea were described in many species of parasitoids in the Trichogrammatidae (Olson and Andow, 1993) or Aphelinidae (Viggiani and Mazzone, 1982). Variability in the length of these sensilla has been described in other Braconidae species (Norton and Vinson, 1974; Roux et al., 2005) but was not clearly observed here. For sensilla placodea, known in many species, electrophysiological studies (Vareschi, 1971) showed that neurones innervated by these sensilla are sensitive to semiochemicals induced by plants (Ochieng et al., 2000). As it was the case for females and males, the authors hypothesized that males used plant-related chemical cues, probably in conjunction with sex pheromones, to locate females. WP type trichodea have been described in other species of parasitoids (Reil, 1999; Miller, 1972;
Slifer, 1969; Steinbrecht, 1997; van Baaren et al., 1999). TP type trichodea are perpendicularly erected from the cuticle and emerge from the layer of other sensilla as it was observed in other species, allowing them to make the first contact with substrates or the sexual partner. Sensilla coeloconica type I have been found in both sexes in different species of Hymenoptera (Altner et al., 1983; Ochieng et al., 2000). Sensilla coeloconica types I and II were found in several other species (see Table 1).

Differences Between Males and Females

Chapman (1982) observed that males of insect species in which sex pheromones were important for mating had generally more olfactory sensilla than females. This has also been observed in *A. rhopalosiphi* where the global number of olfactory sensilla (including placodea, trichodea WP and coeloconica type I) was higher in males than in females (Table 1). This observation, correlated with observations of mating behavior in this species (Bourdais, unpublished data), could mean that the olfactory sense might be involved in female recognition by the male. Host recognition at long distance by females was also linked with this type of sensilla. Bin et al. (1989) suggested that sensilla placodea have different functions in males and females, particularly because of the number of neurones which innervated them. Placodea could thus constitute a receptor of sexual pheromones for males and a receptor for kairomones and other odors present in the environment of the host for females. In *Cotesia* species (Bleeker et al.,
2004; Roux et al., 2005), placodea sensilla are longer in males than in females, which was not the case in *A. rhopalosiphi*. The gustative function is provided by the sensilla trichodea TP and they were more numerous in females than in males in *A. rhopalosiphi*. The gustatory sense is important in the process of host recognition and evaluation, as the choice of the female (accept or reject the encountered aphid) directly affects her fitness. In males, these sensilla are probably linked with courtship behavior. Observations on *A. rhopalosiphi* showed that even if a male is first attracted by a female, the courtship sequence could be stopped at the first contact between the male antennae and any part of the female body (Bourdais, unpublished data). On these sensilla, electrophysiological studies using EAG should be done to understand which cues are perceived by the males.

**Impact of Cold or Heat Exposure**

After a cold exposure, both male and female sensilla organization was modified in all observed individuals. Although it was not statistically demonstrated, it seems that there was only a slight decrease of some types of sensilla (placodea or trichodea WP). We observed the appearance of morphologically abnormal sensilla, but in very small number (less than 15 abnormal sensilla, including all types, in one antenna). For heat exposure, modifications also occurred but the effects seemed to be less intense than those observed for cold exposure (but the duration of heat exposure was shorter).

The differences observed between cold and heat exposures could be explained by the intensity/duration characteristics of the stress. For both stress types, intensity was high because the selected temperatures were at the limit of development of the species, based on preliminary observations. Cold exposure can modify the morphology of individuals by four types of deleterious effects. First, cold exposure of immature instars can induce deleterious effects by freezing, slowing spermato-genesis, inducing male sterility (Rigaux et al., 2000) and ovarian malformations (Hanna, 1935). The second effect is desiccation, which can induce female sterility or low fecundity (Arlian, 1979). The third is starvation because individuals exposed to low temperature at the immature...
instars continue to use resources but are unable to feed (de Bach, 1943). The fourth is an increasing number of genetic alterations (Sehnal, 1991). Although moderate heat is known to increase the rate of biochemical reactions, no study has shown that heat exposure induced morphological alterations. However, heat-induced desiccation could possibly be responsible of the abnormal sensilla. Such a mechanism could explain the differences observed between the two forms of thermal stress. In our study, global morphological alterations (merged antennomeres) were observed on males only. However, the morphological alterations resulting from cold or heat exposure affected only a few sensilla and the major part of the sensory equipment was not significantly modified. Moreover, there was not any large-scale decrease in the number of sensilla after cold or heat exposure. Behavioral experiments made on the same population (Bourdais, unpublished data) show that after cold or heat exposure, the perception of odors was still possible in some individuals because tests of host recognition for females, and of female recognition for males, found that both sexes were more or less able to recognize odors, even if the behavior was not optimal. On the basis of our results, we can hypothesize that the decrease of behavioral performance currently observed after cold storage in parasitoids (van Baaren et al., 2005) is not entirely attributable to modification of sensilla as was first suggested. In the future and in order to understand why abnormal behaviors occur after cold or heat stress, it would be desirable to combine morphological analyses with electrophysiological techniques to determine the stimuli that are communicated to the brain.

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