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Mating behaviour of *Aphidius ervi* (Hymenoptera: Braconidae): The role of antennae

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Abstract. In the mating behaviour of *Aphidius ervi* Haliday the antennae play a pivotal role in partner recognition and acceptance. Mating failure was always observed when antennal contact was experimentally prevented. The male of *A. ervi* has filiform antennae, consisting of scape, pedicel and 18–20 cylindrical antennomeres (flagellar segments), which bear numerous types of sensory structures and, interspersed among the multiporous plate sensilla, especially on the 1th and 2nd flagellar segments, scattered pores. A secretion oozes from these pores in virgin males exposed to conspecific females. Transmission electron microscopy revealed that these pores are the external openings of integumentary glands. Behavioural and morpho-functional observations indicated that a double step sex recognition mechanism is present in *A. ervi*, as in other parasitic Hymenoptera. Basically, female recognition by males appears to be mediated by a volatile sex pheromone, that triggers the behavioural sequence leading to mounting. Then, the female recognizes and accepts the male after antennal contact. This is mediated by the secretion that oozes from the male antennal glands, which acts as a contact pheromone.

INTRODUCTION

In many species of parasitic Hymenoptera, from different families, the antennal glands of males play an important role during courtship behaviour (Dahms, 1984; Bin et al., 1988; Isidoro et al., 1996; Guerrieri et al., 2001). Glandular secretions are released externally and spread onto female antennae by specialized "release and spread sites" (Isidoro et al., 1996). These are peculiar cuticular structures, which can take the form of scales, plates, pegs, carinae or keels, associated with the pores (gland outlets), and are often located on variously modified antennomeres (Bin et al., 1999; Isidoro et al., 1996; Romani et al., 1999; Sacchetti et al., 1999; Guerrieri et al., 2001). However, in some cases the "release and spread sites" may be inconspicuous and localized on unmodified antennomeres. This is the case in *Aylax minor* Hartig (Hymenoptera: Cynipidae), where scattered pores are located laterally on the 3rd antennomere (Isidoro et al., 1999).

Sexual behaviour of *Aphidius ervi* Haliday was described in detail by Mackauer (1969). During pre-mating courtship, males rapidly flick their antennae against those of the female, while during mating only slow flicks are observed. This behaviour, also observed in some Alysiini braconids (Wharton, 1984), suggests the possibility of communication mediated by antennal contact and is the subject of the present paper.

The antennae of male *A. ervi* are not obviously macroscopically modified and/or have specialized structures externally. Based on behavioural and morpho-functional observations, however, this study indicates that antennal glands are present and are possibly involved in partner recognition and acceptance.

MATERIALS AND METHODS

Insects culture

A. ervi was reared on the pea aphid, *Acyrthosiphon pisum* (Harris), maintained on potted broad bean plants (*Vicia faba* L.). The parasitoid culture was kept at 25 ± 1°C, 70 ± 5% r.h. and 16L : 8D photoperiod.

Parasitoids used in the experiments emerged from mummies that were collected from the rearing cage and isolated in glass vials. Freshly emerged adults were fed with a water solution of honey (50% v/v) and kept at room temperature (≅ 25°C).

Mating behaviour

To assess the role and the importance of antennae in the mating behaviour of *A. ervi*, mating was observed, after antennal amputation, according to the following experimental plan:

– male with left antenna removed vs. female with left antenna removed (*same side amputation*);

– male with right antenna removed vs. female with left antenna removed (*opposite side amputation*);

– intact female vs. intact male (*control*).

Observations were continued until at least 10 valid bioassays (i. e. mounting followed or not by mating) of all those combinations were obtained.

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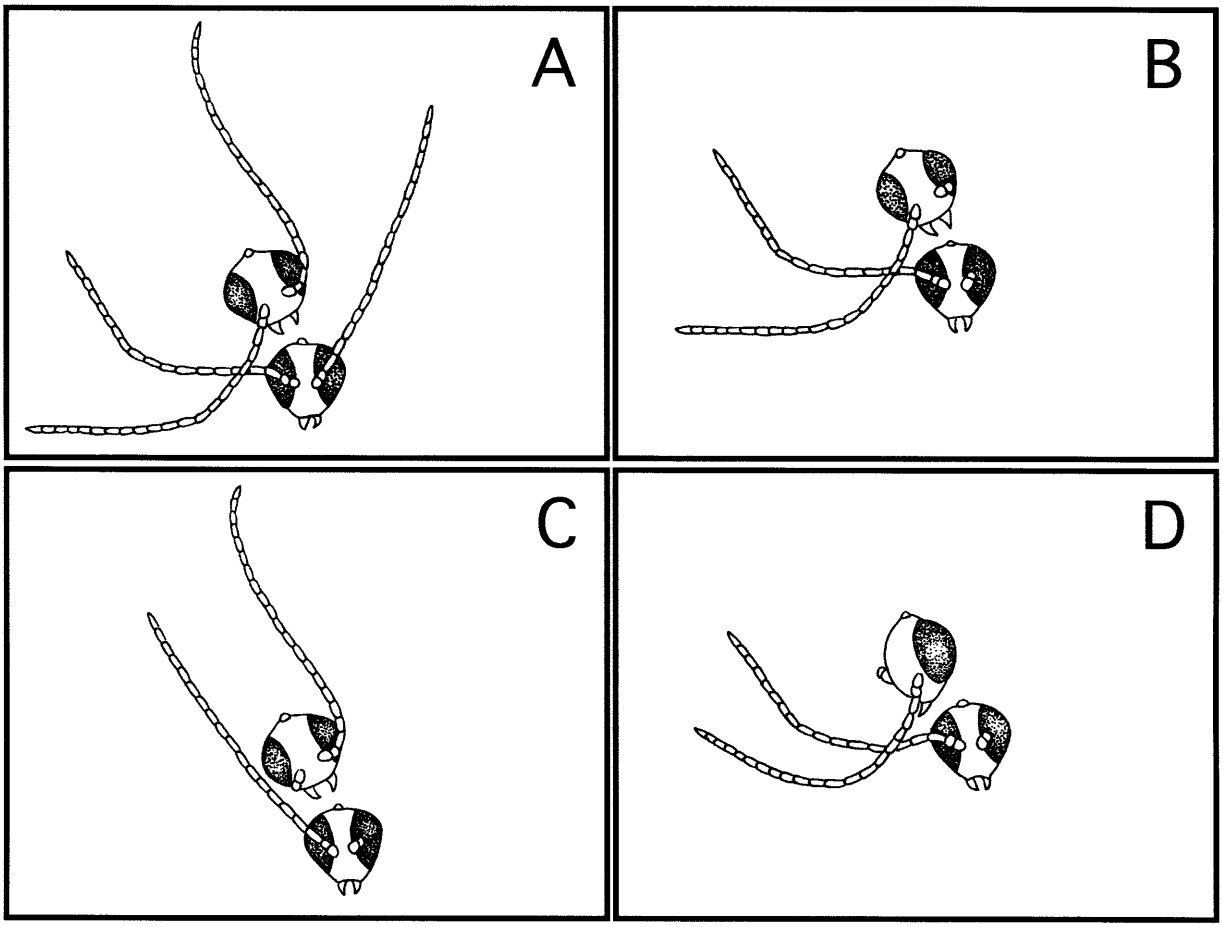

Fig. 1. A – diagrammatic representation of how a male uses its antennae to flick those of a female during pre-mating courtship in *Aphidius ervi*; B – amputation of antennae in both sexes on the same side did not prevent unilateral antennal contact, which in most cases was enough to induce mating; C – amputation on opposite sides normally prevented antennal contact and always resulted in mating failure, except when D – males were able to establish antennal contact by assuming a slightly transverse position.

The antennae were amputated under a stereomicroscope on a cold table (Peltier, Labco®), at 2°C, using a scalpel blade. All the antennomeres, except scape and pedicel, were removed. Parasitoids were kept individually in glass vials until used for mating, which occurred at least 3 hours after antennal amputation. Mating behavior was observed in a glass vial (10 × 75 mm), at room temperature (≅ 25°C), using parasitoids 12–24h after emergence. Each pair was observed for a maximum 10 minutes. The different combinations of antennal amputation were randomly tested in different days in order to reduce any uncontrolled experimental bias.

Mating behaviour was recorded using a JVC VHS Professional Editing Recorder connected to a Moritex micro-Scopeman video-camera.

The incidences of mounting and mating were compared using a chi-square test for two independent samples, and the duration of the different phases of mating behaviour were compared using Student's t -test.

Ultrastructure

For scanning electron microscopy (SEM), 5 *A. ervi* virgin males were beheaded and their heads immediately immersed in a 50% ethanol solution. In addition 5 males were identically processed soon after they had been exposed to conspecific females and had started wing fanning. After dehydration in a graded ethanol series, the heads with antennae were critical point dried (in a Balzers Union CPD 020 unit), gold coated (in a Balzers Union SCD 040 unit), and examined with a Philips XL 30.

For transmission electron microscopy (TEM), 5 males of *A. ervi* were anaesthetized with CO2 and immediately immersed in a 2,5% glutaraldehyde solution in 0.1 M cacodylate buffer, containing 5% (w/v) sucrose, pH 7.2. Then the antennomeres to be processed were detached to facilitate the penetration of fixative, and kept at 4°C for 2h. After rinsing overnight in cacodylate buffer, the specimens were postfixed in 1% osmium tetroxide at 4°C for 1h and rinsed in the same buffer. Dehydration in a graded ethanol series was followed by embedding in Epon-Araldite with propylene oxide as bridging solvent. Thin sections were cut with a diamond knife on a L.K.B. "Nova" ultramicrotome, and mounted on collodium-coated 50 mesh grids. Finally, the sections were observed in a Philips EM 400T, after staining with uranyl acetate (20 min, room temperature) and lead citrate (5 min, room temperature).

RESULTS

Mating behaviour

The mating behaviour of intact insects and those that had an antenna amputated was the same. It was similar to the description provided by Mackauer (1969), but the duration of pre-mating interval was much shorter (25 ± 13.8 sec vs. 146.3 ± 96.5 sec). A male became excited just after introduction into a glass vial containing a virgin

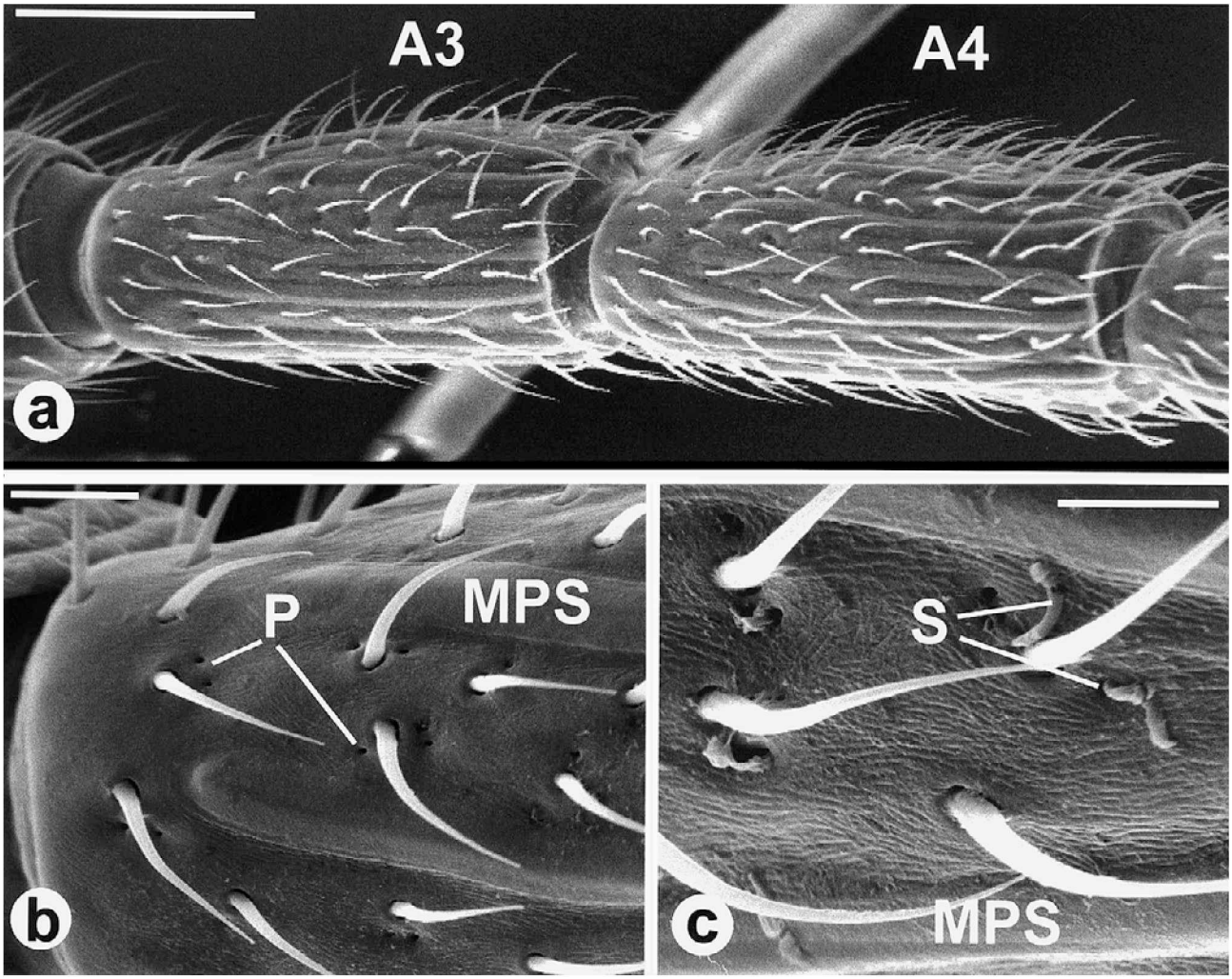

Fig. 2. *Aphidius ervi* scanning electron micrographs. a – lateral view of male A3 and A4 (bar = 50 μm); b – male A3 showing the presence of several surface pores (bar = 5 μm); c – which ooze secretion when males are exposed to conspecific females (bar = 2.5 μm). Abbreviations used: A3 and A4 – third and fourth antennomeres respectively; MPS – multiporous plate sensilla; P – pores; S – secretion.

female and almost immediately started intense wing fanning. When a male encountered a female, which very often ran away, the male tried to catch and mount her. Even when successfully mounted a female still tried to walk away and to dislodge the male by rapid head and abdominal movements, and by kicking with her hind legs. However, this resistance quickly subsided when the male started to flick its antennae against those of the female (Fig. 1A), and mating followed. Based on the use of antennae, mating can be divided in two phases. In the first phase the male slowly and gently simultaneously touches its partner's antennae with both his antennae. In the second phase, the male stays motionless with his antennae up.

In most cases mating occurred after mounting in intact control insects. In fact, only one male out of 28 was repeatedly dismounted and eventually failed to mate (Tab. 1). *A. ervi* males that had one antenna removed exhibited the normal intense wing fanning in the presence of a virgin female, but mounting of a partner was significantly impaired, compared to intact controls ($df = 1$, $\chi^2 = 15.2$; $P < 0.001$). However, in the "*same side amputation*" experiment, when mounting occurred, standard courtship behaviour was observed. In this case, the male regularly touched the right antenna (Fig.1B) of the female with his right antenna which usually triggered the complete mating sequence. In fact, only 4 males out of 14 were dismounted, while the others were able to arrest the female by unilateral antennal contact and to mate (Tab. 1). Nevertheless, this type of antennal amputation affected mating success, which was significantly lower than in intact insects ($df = 1$, $\chi^2 = 5.35$; $P < 0.05$).

TABLE 1. Number of pairs tested (N), and number of mountings and successful matings recorded for each experimental condition.

	N	Mounting	Mating
Control	40	28	27
Same side amputation	42	14	10
Opposite side amputation	33	10	2

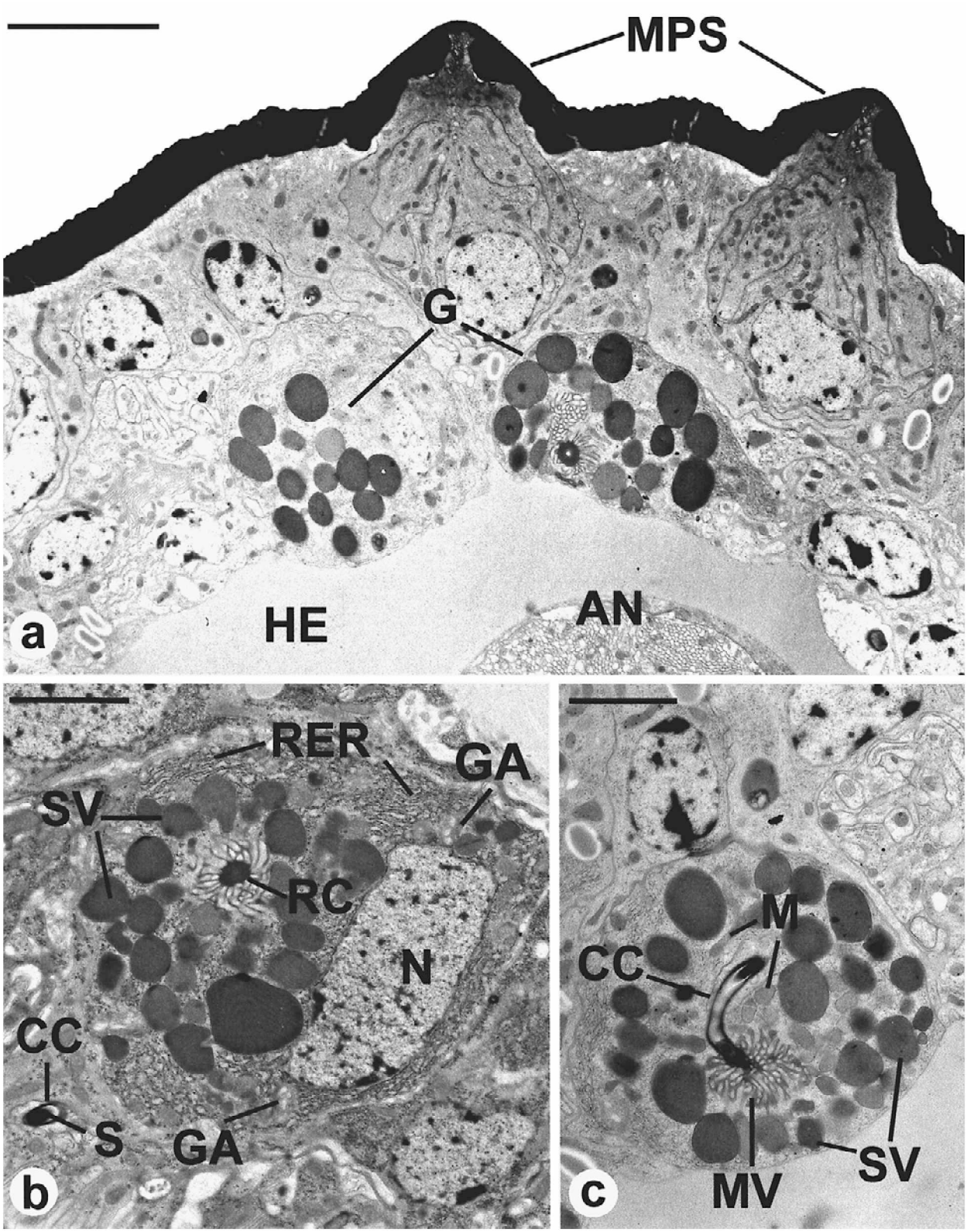

Fig. 3. *Aphidius ervi* transmission electron micrographs. a – male A3 cross section showing two glandular units underneath the multiporous plate sensilla (bar = 5 μm); b – perinuclear region of a secretory cell showing a well developed nucleus, large rough endoplasmic reticulum, and Golgi complexes with numerous secretory vesicles (bar = 2.5 μm); c – apical region of a secretory cell showing electron-dense vesicles surrounding the receiving canal (bar = 2.5 μm). Abbreviations used: AN – antennal nerve; CC – conducting canal; A3 – third antennomere; G – glandular units; GA – Golgi apparatus; HE – haemocoel; M – mitochondria; MPS – multiporous plate sensilla; MV – microvilli; N – nucleus; RER – rough endoplasmic reticulum; RC – receiving canal; S – secretion; SV – secretory vesicles.

TABLE 2. Length of pre-mating and mating intervals (in seconds mean ± standard deviation). See text for description of considered behavioural steps.

	N	Pre-mating	Mating	
			phase 1	phase 2
Control	27	25 ± 13.8	26.3 ± 39.4	27.7 ± 14.3
Same side amputation	10	27.6 ± 13.4	21.0 ± 9.0	25.1 ± 14.4

In the case of "*opposite side amputation*", males on mounting tried to start the courtship behaviour, but antennal contact was prevented (Fig. 1C). Only 2 males out of 10 mated. In both cases the males assumed a slightly transverse position, which allowed cross-antennal contact (i.e. right-left antennal contact) with the female (Fig. 1D). All the other males were rapidly and repeatedly dismounted. The negative effect on mating of "opposite side amputation" was evident. In this case, mating success was significantly lower than in insect with "same side amputation" ($df = 1$, $\chi^2 = 6.16$; $P < 0.05$).

The duration of the different steps of mating behaviour described above was not significantly affected by antennal amputation (Tab. 2).

Ultrastructure

A. ervi males have filiform antennae (Fig. 2a), consisting of 20–22 antennomeres which are here consecutively numbered starting from the scape (A1). This follows the system used by Isidoro et al. (1996) in a similar study on the functional anatomy of antennae in Hymenoptera. SEM revealed the occurrence of scattered pores, interspersed among the multiporous plate sensilla on several antennomeres (Fig. 2b). These pores are more numerous on the lateral surfaces of A3 and A4. A paste-like secretion oozed from these pores when virgin males were exposed to conspecific females (Fig. 2c), but not when kept isolated (Fig. 2b).

Serial transverse and longitudinal thin sections of A3 and A4 of males show the presence of numerous adjacent integumentary glands, located underneath the cuticle (Fig. 3a). These are isolated bicellular glands consisting of an outer canal cell and an inner secretory cell. The secretory cell has a voluminous regular shaped nucleus (Fig. 3b) located toward the base of the cell. The cytoplasm contains numerous mitochondria and is filled by a large rough endoplasmic reticulum and well developed Golgi complexes, releasing numerous electron-dense secretory vesicles (Fig. 3b, c), which indicates intense secretory activity. The basal part of the cell membrane has relatively small invaginations, while the apical part is surrounded by a microvillar border, which delimits a porous receiving canal, connected to the conducting canal (Fig. 3b, c). The canal cell has a reduced cytoplasm enclosing the conducting canal, which reaches the external pore openings.

CONCLUSIONS AND DISCUSSION

The descriptions of mating in *A. ervi* and related *Aphidius* species, such as *Aphidius pisivorus* Smith, *Aphidius smithi* Sharma & Subba Rao (Mackauer, 1969) and *Aphidius nigripes* (Ashmead) (McNeil & Brodeur, 1995), indirectly suggest that the antennae of males play a pivotal role in mating. The observations reported in the present study provide experimental evidence of that role. The antennae of *A. ervi* males have glands on several antennomeres, which are more abundant on the basal flagellar segments. The structures for the release and spread of the secretions from these glands are inconspicuous, not associated with special external structures, and appear to consist of scattered pores, as in *Aylax minor* Hartig (Isidoro et al., 1999). A similar situation is observed in two species of Alysiini braconids, *Alysia alticola* Ashmead and *Aphereta pallipes* Say (Bin et al., unpublished). A paste-like secretion oozes from these antennal pores when a virgin *A. ervi* male encounters a conspecific female and becomes excited, as indicated by the intense wing fanning behaviour that typically precedes mounting. This antennal secretion is produced just prior to antennal contact. Antennal contact, even unilateral, as indicated by the "*same side amputation*" experiments, is an essential step in the mating behaviour. This is further corroborated by the fact that the "*opposite side amputation*" normally prevented mating, except when the males, as in two cases, assumed an unusual transverse position that allowed antennal contact.

These behavioural and morpho-functional observations strongly indicate that antennal glands produce a secretion that acts as a contact pheromone, which elicits male acceptance, and that the emission of this secretion is stimulated by the presence of a female. The isolation and characterization of the active components of this pheromone will provide evidence in support of this view and offer interesting opportunities for comparative studies, which may provide new insights into the evolutionary mechanisms involved in population isolation and differentiation in Aphidiinae.

In conclusion, the mechanism controlling partner acceptance during the mating behavioural sequences appears to nicely fit into a more generalized strategy that includes two sequential steps. Female recognition by males triggers the behavioural sequence leading to mounting, and is mediated by a sex pheromone, whose occurrence is widely reported in parasitic Hymenoptera (Rao & DeBach, 1969; Kimani & Overholt, 1995; Pompanon et al., 1997; Jewett & Carpenter, 1999), including Aphidiinae (Powell & Zhang, 1983; Bouchard & Cloutier, 1985; Pennacchio & Tremblay, 1987; Decker et al., 1993; McNeil & Brodeur, 1995; Nazzi et al., 1996). Females recognize and accept males after antennal contact. This is mediated by males producing a contact pheromone. The production of a contact pheromone by males of parasitic Hymenoptera appears to be a widespread phenomenon, as the males of many species, from different families, have antennal glands and antennation during precopulatory courtship, triggering partner acceptance and mating, is common (Dahms, 1984; Bin et al., 1988; Isidoro et al., 1996; Guerrieri et al., 2001).

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