A finely tuned strategy adopted by an egg parasitoid to exploit chemical traces from host adults

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SUMMARY

Scelionid egg parasitoids can obtain reliable information on the presence of host eggs by discriminating host gender on the basis of chemical footprints of their co-evolved hosts, with a strong preference for the footprint left by host females. Based on the concept of dietary specialization and infochemical use in natural enemies, it could be predicted that host gender discrimination in specialist species belonging to the genus *Trissolcus* is further tuned to specific cues from distinctive chemical traces left by host females as a consequence of copulation and/or oviposition. To test this hypothesis we used the system *Murgantia histrionica –
Trissolcus brochymenae*. Our results showed that the females of the egg parasitoid search intensely on chemical traces left on the substrate by host females that had mated but had not yet laid host eggs compared with the chemical traces left by virgin or parous host females. This preference for mated females that had not yet laid host eggs was strictly related to the transfer of sperm and associated substances from males to females during copulation. The compounds that mediated the arrestment response of *T. brochymenae* females are part of the host cuticle, and those that play a role as gender-specific cues seemed to be present in the legs of the host adult. This result represents an interesting new piece of information regarding the exploitation of indirect host-related cues by egg parasitoids. It reveals the existence of a finely tuned strategy that allows the parasitoid to find newly laid host eggs, as chemical traces left by mated host females that have not yet laid eggs are strongly correlated with the moment of oviposition.

Key words: *Trissolcus brochymenae*, *Murgantia histrionica*, egg parasitoid, indirect host-related cues, arrestment response.

INTRODUCTION

Hymenopteran parasitoids represent a large and varied group of insect species that have ecological and economic relevance in influencing and/or controlling populations of herbivorous insects in natural and agricultural ecosystems. To locate their hosts in a multitrophic context and complex environment, female wasps can use a wide range of stimuli; among them, the chemical cues, named semiochemicals, play a key role (Vinson, 1985; Vet and Dicke, 1992). Additionally, the quality of the host strongly affects parasitoid fitness in terms of development time, survival to the adult stage, size and fecundity (Godfray, 1994). Therefore, for insect parasitoids, hosts represent a limited resource as their nutritional status can decay rapidly over time. Consequently, parasitoids that utilize chemical cues to efficiently find and exploit high quality hosts improve their fitness (van Alphen and Vet, 1986; Godfray, 1994).

The quality of eggs can decrease rapidly with age (as embryo development proceeds) so egg parasitoids prefer to lay in fresh eggs (Strand, 1986; Vinson, 1998). To cope with such limited time windows of host availability, egg parasitoid females have evolved specific strategies to exploit direct host-related cues associated with the presence of suitable host eggs (Romeis et al., 2005; Hilker and Meiners, 2006; Fatouros et al., 2008). For example, in studies with the egg parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae), it was shown that female wasps most successfully accept the eggs of their main host, *Nezara viridula* (Heteroptera: Pentatomidae), when they are newly laid, up to approximately 72h old (Wilson, 1961; Bin et al., 1993). Follow-up investigations showed that *T. basalis* responds to volatile synomones emitted by bean leaves onto

which an egg mass has been oviposited (Colazza et al., 2004). However, the production of such oviposition-induced synomones is influenced by the age of the eggs. In fact, bean leaves bearing eggs that are 72–96h old still attract the parasitoid whereas leaves bearing hatched eggs (~120h old) do not (Colazza et al., 2004). Similar evidence has been found for the females of *Oomyzus gallerucae* (Hymenoptera: Eulophidae), the egg parasitoid of the elm leaf beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae) (Hilker and Meiners, 2006). Plants can also produce contact synomones as a consequence of herbivore egg deposition and these direct host-related cues are perceived by parasitoids only when they walk on the damaged plants (Conti et al., 2006; Fatouros et al., 2008). In addition, a wasp's arrestment response to contact synomones is influenced by the age of the eggs, as has been shown for egg parasitoids attacking some pests of cabbage plants, e.g. *Trichogramma brassicae* and *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae), parasitoids of *Pieris brassicae* (Lepidoptera: Pieridae) (Fatouros et al., 2005; Fatouros et al., 2007), and *Trissolcus brochymenae* Ashmead (Hymenoptera: Scelionidae), a parasitoid of *Murgantia histrionica* Hahn (Heteroptera: Pentatomidae) (Conti et al., 2006).

These findings indicate that direct host-related chemical cues provide egg parasitoid females with reliable information on the presence of host eggs of a suitable age. However, during the host location process, egg parasitoid females can also 'eavesdrop' on chemical cues arising from the activities of stages of the host other than the eggs. Examples of such indirect host-related cues are host pheromones and allomones, the kairomones from the scales of adult

Lepidoptera or the kairomones from the traces left behind by adult pentatomids while moving on the plant (for a review, see Fatouros et al., 2008). The ability to exploit indirect host-related cues is broadly adopted by egg parasitoid females (Vinson, 1998; Fatouros et al., 2008). These cues mainly enable egg parasitoid females to arrive in the vicinity of host eggs on the plant. However, as opposed to direct host-related cues, indirect host-related cues could be less reliable indicators of the age of egg, as is the case of wasps that exploit the sex pheromones of herbivores that oviposit far from the mating sites (Powell, 1999). Other indirect host-related cues can endow foraging parasitoids with more detailed information concerning the physiological condition of the hosts, such as gravid females contaminated by the volatile anti-aphrodisiac pheromone that becomes more attractive for phoretic egg parasitoids (Fatouros et al., 2005; Fatouros et al., 2008). However, spying on host sexual signals may not be effective for non-phoretic egg parasitoid females as was demonstrated, for example, for two egg parasitoids, one phoretic and the other non-phoretic, that attack the same host *Podisus maculiventris* (Heteroptera: Pentatomidae) (Bruni et al., 2000). Females of the phoretic egg parasitoid *Telenomus calvus* (Hymenoptera: Scelionidae) respond to the synthetic pheromone of the host whereas females of the non-phoretic egg parasitoid *Telenomus podisi* (Hymenoptera: Scelionidae) do not (Bruni et al., 2000).

Among the possible indirect host-related cues, the chemical residues left on a substrate by host adults while moving around play a relevant role. The main evidence for this phenomenon comes from studies of pentatomid insects and their egg parasitoids, such as *N. viridula – T. basalis* (Colazza et al., 1999; Peri et al., 2006), *M. histrionica – T. brochymenae* (Conti et al., 2003; Conti et al., 2004), *Eurydema ventrale* (Heteroptera: Pentatomidae) – *Trissolcus simoni* (Hymenoptera: Scelionidae) (Conti et al., 2004) and *Euschistus heros* (Heteroptera: Pentatomidae) – *Telenomus podisi* (Borges et al., 2003). Once in contact with host footprints, egg parasitoids adopt a motivated searching behavior characterized by a variation in locomotion activity, which stimulates them to intensively search on patches where footprints are present (Colazza et al., 1999; Borges et al., 2003; Conti et al., 2003). Such prolonged searching increases the probability of locating host eggs; this seems especially critical for parasitoids that attack host species that are polyphagous and normally lay their eggs far from the mating site (Colazza et al., 1999; Fatouros et al., 2008). Under such circumstances, cues associated with host males would be of minimal use to egg parasitoids because these cues would not help the parasitoids to locate eggs. Instead, egg parasitoid females should optimize their sensitivity to cues produced by host females. The available data supports this scenario, because egg parasitoid females demonstrate the ability to discriminate between chemical residues left by females and males of their pentatomid hosts (Colazza et al., 1999; Conti et al., 2004).

A prediction of the concept of dietary specialization and infochemical use in natural enemies is that specialist species' use specific cues more frequently than generalist species' (Vet and Dicke, 1992; Steidle and van Loon, 2003). Therefore, consistent with this concept, egg parasitoid species with a narrow host range should have developed a strategy finely tuned to exploit traces left by host females in a physiological condition that could ensure access to newly laid eggs.

To test this prediction, a series of experiments were developed to investigate whether the exploitation of host chemical footprints by an egg parasitoid is influenced by the physiological condition of the host. The Nearctic association *T. brochymenae – M.*

histrionica study model was used; this association qualifies as a 'specialist at host/prey and nearly at host plant/feeding substrate level' (*sensu* Vet and Dicke, 1992; Steidle and van Loon, 2003). In fact *T. brochymenae* has been recorded from 11 pentatomid species belonging to the same family (Salerno, 2000), and the harlequin bug *M. histrionica* attacks only Brassicaceae and Capparidaceae (McPherson, 1982).

In this paper, first we investigated the ability of the egg parasitoid to discriminate host gender on the basis of the host's varied physiological condition, i.e. virgin adults, mated adults or mated parous females. We will demonstrate that female wasps prefer footprints from mated host females but only when they had not yet laid eggs. Second, we studied the role of host mating, which affects the parasitoid's preferences for the traces left by mated females. We will show that the wasp's preference for mated females is strongly associated with the full transfer of sperm and associated substances to the female during copulation. Third, we used dissected body parts from mated host adults to study the origin of the contact kairomone. We will provide evidence that compounds mediating the arrestment of *T. brochymenae* females are derived from the host cuticle, and that compounds enabling female wasps to distinguish between mated males and mated females are present on the host legs.

MATERIALS AND METHODS
Insect colonies
Harlequin bugs (*M. histrionica*) were originally collected from cabbage in the Beltsville area, MD, USA in 2000. Adults of *T. brochymenae* were obtained from *M. histrionica* eggs laid on *Isomeris arborea* Nutt. (Capparidaceae) in San Diego, CA, USA in 2000. Both insects were maintained in quarantine conditions in a growth chamber at $25\pm1°C$, $60\pm5\%$ RH, 16h:8h light:dark at the Entomology laboratories of the University of Perugia, Perugia, Italy. The colony of *M. histrionica* was reared in plastic cages and fed cabbage as described by Conti et al. (Conti et al., 2004). Every 2–3 days, nymphs of the last instar were individually isolated by gently transferring them using a fine brush into a single plastic pot (Ø=40mm, height=65mm), and providing them with water and a portion of cabbage. These nymphs were observed daily until adult emergence, so that individuals of known ages were continuously available for the experiments. The colony of *T. brochymenae* was reared on eggs of *M. histrionica*, and adult wasps were kept in 85ml glass tubes and fed small drops of Safavi diet (Safavi, 1968). After emergence, male and female parasitoids were kept together to allow mating. In all of the experiments, female wasps were 2–3 days old, mated and naïve for adult host chemicals. Female wasps used for experiments were individually isolated in small vials 16–17h before the bioassays and allowed to acclimatize in the bioassay room for at least 30min before bioassays.

Bioassays procedure
All behavioral assays were conducted in open arenas consisting of rectangular filter paper sheets (340×240mm). In the center of the open arena, a 6cm diameter circle (2827mm², representing about 3.5% of the entire arena surface) was treated with the test stimuli described below, while the rest of the arena was left untreated. Arenas not contaminated were used as controls. A single female wasp was gently released onto the center of the treated area. Wasps that soon after their release displayed typical arrestment responses, i.e. with the antennae held in contact with the arena surface, were scored as 'response'. Wasps that did not show the arrestment response were recaptured and retested approximately 1min later.

After three unsuccessful trials, wasps were considered 'non-responsive'. The movements of the parasitoids over the entire arena were recorded with a video tracking and motion analysis system (for details, see Colazza et al., 2007). The time spent by female wasps in the whole arena until they flew away or walked off was scored as the arena 'residence time' (s). This parameter properly describes the wasp searching behavior, which is characterized by returning several times to the treated area followed each time by an examination of the surface around the treated area. When doing a comparative bioassay, the different treatments were tested on several days and they were alternated on the same day after having tested a group of wasps for each arena. All experiments were carried out from 09:00 h–14:00 h, in an isolated room at 26±1°C, with the arena illuminated by two 180 mm-long fluorescent lights (full spectrum 5900 K, 11 W; Lival, Sipoo, Finland).

Parasitoid discrimination of host sex as a function of physiology

In order to examine the wasp's ability to discriminate between host genders, females of *T. brochymenae* were exposed to open arenas contaminated with traces left by adults of *M. histrionica* in the following combinations: (1) virgin females *vs* virgin males; (2) mated females *vs* mated males; and (3) mated parous females (one egg mass laid) *vs* mated males. Open arenas not contaminated were used as controls. Virgin adults were obtained from adults that were kept individually separated from the time of emergence. Mated adults were obtained from pairs that had copulated and then, immediately after mating, were separated and kept individually isolated for 24 h before the experiment. Mated parous females were obtained from mated females that were kept isolated after mating and checked regularly until they had deposited their first clutch of eggs and were then used for the experiments approximately 24 h later. Virgin adults and mated adults were used in the bioassays at the age of approximately 10–14 days post-emergence. The age of mated parous females was variable as the time span between mating and oviposition is not uniform for this species (G.S., F.F. and E.C., personal observation). In all cases, parous females that were older than 15 days were not used in our experiments. The adults used were kept isolated, as described above, in plastic pots (Ø=40 mm, height=65 mm) with nylon mesh on the top before being tested in the bioassays. To contaminate an open arena, a single adult for each gender and physiological condition was kept in place for 1 h and forced to walk on the filter paper as described by Conti et al. (Conti et al., 2003). Both uncontaminated and contaminated open arenas were used to test five responding female wasps, and the experiment was repeated four times (20 replications) for a total of 140 tested wasps.

Parasitoid responses to traces left by host adult females with interrupted copulation

In order to examine the influence of mating on the response of *T. brochymenae* to traces of *M. histrionica* adults, female wasps were exposed to open arenas contaminated with traces left by mated host females with interrupted copulation at different time intervals *vs* mated host females. Open arenas not contaminated were used as controls. Mated host females with interrupted copulation were obtained from adult pairs that were allowed to mate and then gently separated after 0.5, 2, 4 or 8 h. The time interval of 8 h represents about a third of the mean duration of the first copulation in our laboratory conditions (G.S., F.F. and E.C., personal observation). Mated females were obtained from pairs that ended the copula. All of the host adults used for the bioassays were kept isolated for 36 h

before the experiment in plastic pots (Ø=40 mm, height=65 mm) with nylon mesh on the top. A single host female was used to contaminate an open arena as described above, and five wasps were tested for each treated and untreated arena. The experiment was repeated four times for a total of 120 tested wasps.

Presence of sperm and associated substances in the spermathecal bulb and the dilated portion of the spermathecal duct of host adult females with interrupted copulation

Mated females of *M. histrionica* with interrupted copulation and mated females, after having been used for the experiment previously described, were killed by freezing at –18°C for about 30 min and were dissected in saline solution under a binocular stereo microscope (Leica DMLB, Milano, Italy). Then, the spermathecal bulb and the dilated portion of spermathecal duct (Pendergrast, 1957) were evaluated for the presence of sperm and associated substances. Moreover, the width of the dilated portion of spermathecal duct was measured using a micrometric optic device (Leitz, Wetzlar, Germany 16×) fitted to the stereo microscope. Four individuals were dissected for each condition for a total of 20 dissected adults. Digital pictures were obtained using a high-resolution digital camera (Leica DC 300F).

Parasitoid responses to dissected body parts from mated host adults

Mated adults of *M. histrionica*, frozen at –18°C for about 30 min, were dissected into scutellum and legs. The scutellum was selected as a control body part, because it would not be contaminated with any compounds from the legs during grooming behaviors. Fine scissors were used to clip off the legs at the coxa level and cut off the scutellum from the pronotum. A single open arena was treated by wiping with 18 legs and three scutella dissected from either three mated females or three mated males of *M. histrionica* using fine forceps. In particular the tarsi were gently rubbed for about 1 s on the filter paper to simulate the insect walking (30 times per each tarsum). The arenas contaminated by scutella were obtained by rubbing each scutellum for about 30 s. Arenas not contaminated were used as controls. Each open arena, treated and untreated, was used to test 15 wasps, and the experiment was repeated twice (30 replications) for a total of 150 tested wasps.

Parasitoid responses to hexane extracts of dissected body parts from virgin and mated host adults

Virgin and mated adults of *M. histrionica*, frozen at –18°C for about 30 min, were dissected into entire legs clipped off at the coxa level, wings, pronotum and scutellum under a binocular stereo microscope (Leica DMLB). The inside of the pronotal cuticle was cleaned as thoroughly as possible to remove traces of adhering tissues. Dissected body parts from three adults were weighed, then placed together in 15 ml glass vials and extracted with 10 ml of hexane at 30°C for 2 h. After removal of the body parts, the resulting extracts were evaporated under a gentle nitrogen stream and redissolved in 1 ml hexane (Chomasolv®, Sigma Aldrich, St Louis, MO, USA) and stored at –18°C until assayed. Hexane extracts were tested at doses of one dissected adult equivalent (DAE) by pipetting 333 μl of extracts onto the treatment area of an open arena. The extract was applied as drops, resulting in a patch distribution all over the arena. Open arenas treated with 333 μl of pure hexane were used as controls. The experiments were repeated three times, and each arena was tested with 15 female wasps (45 replications) for a total of 225 tested wasps.

Statistical analysis

The arena residence times of the wasps were evaluated for their normality and normalized through logarithmic transformation when required. Student's t-test for independent samples was used to compare the time spent by female wasps in contaminated vs control arenas. The same test was applied to compare the parasitoid residence time on host females vs host males arenas treated with traces, dissected body parts and hexane extracts (Zar, 1999). The residence times of the wasps in the arenas contaminated by females with interrupted copulation at different time intervals were analyzed by one-way analysis of variance (ANOVA) (Zar, 1999). Due to a lack of significance, the data from the different time intervals were pooled and compared with mated females and with controls, using one-way ANOVA and the unequal N Tukey HSD test for multiple comparisons between the means. All statistical analyses were performed using Statistica 6.0 [Statsoft, 2001, Vigonza (PD), Italy].

RESULTS

Parasitoid discrimination of host sex as a function of physiology

All of the *T. brochymenae* females used for the bioassays showed arrestment responses when exposed to arenas treated with adults of *M. histrionica*. The residence time of wasps was higher on treated arenas compared with untreated arenas (t=15.26; d.f.=138; P<0.001). Then, the ability of the wasps to discriminate between residues left by males or females was significantly affected by the physiological status of the host adult (Fig. 1). Female wasps exploring open arenas contaminated by chemical traces left by virgin adults did not display significant differences between the sexes in the arena residence time (t=0.86; d.f.=38; P=0.397). Instead, female wasps exploring open arenas contaminated by traces from mated adults spent a significantly longer time on the traces left by mated females (t=3.22; d.f.=38; P=0.003). Furthermore, female wasps did not show significant differences between the sexes when exploring open arenas contaminated with traces left by mated parous females or mated males (t=0.77; d.f.=38; P= 0.445).

Parasitoid responses to traces left by host adult females with interrupted copulation

Wasps showed arrestment responses when tested on arenas treated with chemical traces from females with interrupted copulation (Fig. 2). However, the responses of the wasps were not significantly different when they were in contact with traces left by host females with the copula interrupted at different intervals (0.5 h=223±25 s; 2 h=262±42 s; 4 h=241±38 s; 8 h=256±28 s; F=0.29; d.f.=3, 76; P=0.832). By contrast, pooled residence times on arenas contaminated by mated host females with interrupted copula were significantly shorter compared with those obtained in the case of mated host females that ended the copula. Moreover, the residence time spent by parasitoid females on both treated arenas was higher compared with the control (F=130.81; d.f.=2, 120; P<0.001) (Fig. 2).

Presence of sperm and associated substances in the spermathecal bulb and the dilated portion of the spermathecal duct of host adult mated females

The sperm and associated substances were found only in the spermathecal bulb and in the dilated portion of the spermathecal duct of mated adult host females but not in females with interrupted copulation. In fact, under the microscope, the spermathecal bulb of mated host females appeared markedly less transparent and filled with sperm (Fig. 3). The mean width of the dilated portion of the spermathecal duct in mated females vs females with interrupted copulation was not significantly different (1.97±0.23 mm in mated host females and 1.72±0.05 mm in host females with interrupted copula, t=1.72; d.f.=11; P=0.165). However, under the microscope, the dilated portion of the duct appeared swollen and whitish only in mated females, as a consequence of the presence of the associated substance transferred from the male (Fig. 3).

Parasitoid responses to dissected body parts from mated host adults

Female wasps showed arrestment responses when exposed to arenas treated by rubbing tarsi and scutella dissected from *M. histrionica* mated adults. The time spent by *T. brochymenae* females was significantly higher on treated arenas compared with the control (t=10.04; d.f.=148; P<0.001). The arena residence time of the wasps was significantly affected by host gender and body parts. In fact, wasps stayed longer on the arenas treated with tarsi dissected from mated females than on arenas treated with the analogous body parts dissected from mated males (t=–2.38; d.f.=56; P=0.020). There were no significant differences in parasitoid responses towards host males and females when the arenas were contaminated by scutella (t=–0.14; d.f.=58; P=0.893) (Fig. 4).

Parasitoid responses to hexane extracts of dissected body parts from virgin and mated host adults

Consistent with previous results, *T. brochymenae* females showed arrestment responses when exposed to arenas treated with hexane extracts of dissected body parts from virgin and mated host adults. The parasitoid residence time on arenas treated with hexane extracts was higher than on arenas treated with pure solvent (t=16.98; d.f.=223; P<0.001). No significant differences in arena residence
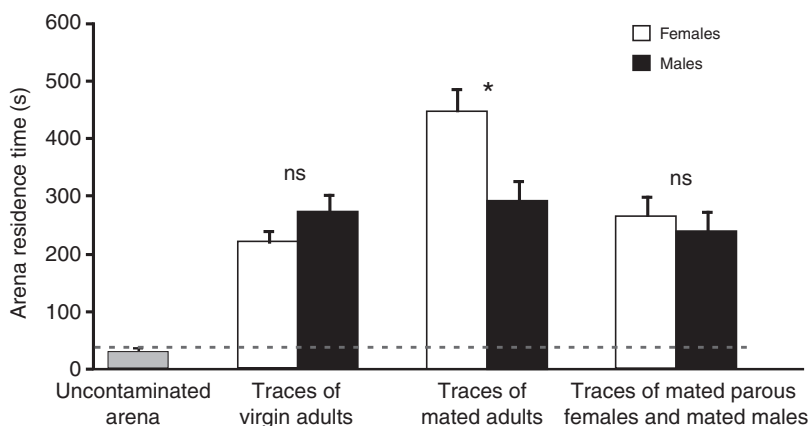

Fig. 1. Residence time (s) of *T. brochymenae* on uncontaminated arenas and on arenas contaminated with chemical traces left by virgin and mated *M. histrionica* males and females and by mated parous females and mated males. Values below the broken line show a lack of arrestment response. Bars indicate means ± s.e.m. (*P<0.01; ns, not significant; Student's t-test for independent samples).
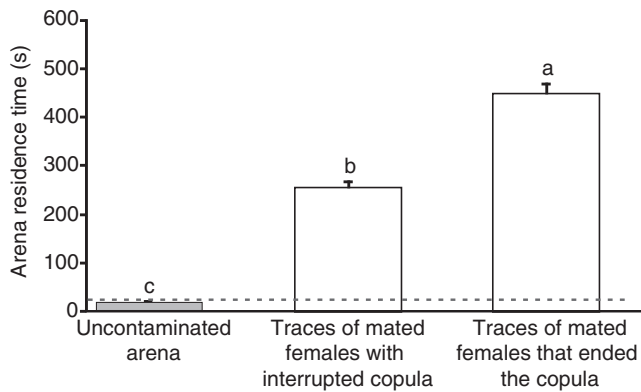
Fig. 2. Comparison among residence times (s) of *T. brochymenae* on uncontaminated arenas and on arenas contaminated with traces left by females with interrupted copula (data from different time intervals pooled) and with traces left by mated females. Residence time (s) on uncontaminated arenas is used as a control. Values below the broken line show a lack of arrestment response. Bars indicate means ± s.e.m. Different letters above the bars indicate significantly different means at *P*<0.001 (ANOVA, Tukey unequal *N*., HSD).

times were shown by female wasps in open arenas treated with extracts from virgin host adults (t=0.81; d.f.=88; *P*=0.418). Instead, there was a significantly longer arena residence time on the arenas treated with hexane extracts from mated females in comparison with mated males (t=3.45; d.f.=88; *P*<0.001) (Fig. 5).

DISCUSSION

The behavioral data provided in this paper confirm the host gender discrimination ability of *T. brochymenae* (Conti et al., 2003; Conti et al., 2004). However, such an ability is expressed only by presence of chemical traces left by mated females that have not yet laid eggs. In all probability, this finely tuned strategy allows the parasitoid to find newly laid host eggs, as chemical traces left by mated host females that have not yet laid eggs are strongly correlated with the moment of oviposition. We might even suggest that this strategy could be considered as a form of ambush [*sensu* Vinson (Vinson, 1998)], because in this way *T. brochymenae* could be in the condition of reaching an area where host eggs are expected to be laid.

The ability to discriminate the host gender was evidenced in another *Trissolcus* species, the egg parasitoid *T. basalis* (Colazza et al., 1999; Peri et al., 2006; Colazza et al., 2007). However, when considering the spatial correlation with the moment of the oviposition, *T. basalis* females seem to adopt a less tuned host gender

discrimination strategy, as they prefer host female traces rather than male traces at all physiological conditions, i.e. virgin and mated (Colazza et al., 1999). Such dissimilarity in these two egg parasitoids *T. basalis* and *T. brochymenae* may be explained in terms of dietary specialization. In fact, *T. basalis* should be included in the group 'generalist at the host and the host plant/feeding substrate levels' (Vet and Dicke, 1992; Steidle and van Loon, 2003), as it was recorded from about 90 species of Pentatomidae and Scutelleridae, and *N. viridula* is extremely polyphagous (Todd, 1989). These two parasitoid species also show different strategies to use direct and indirect host-related cues, because *T. basalis* can exploit volatile oviposition-induced synomones produced within approximately 72 h from oviposition and host traces to restrict the searching area (Colazza et al., 2004), while *T. brochymenae* does not use volatile oviposition-induced synomones but uses volatile compounds from the host and reaches the egg masses at the right age by exploiting chemical traces from mated host females and/or oviposition-induced synomones perceived upon contact (Conti et al., 2003; Conti et al., 2006). Thus, the following scenario is suggested: these egg parasitoids use host pheromones as a good indicator of the presence of a species at long-range, they then exploit contact indirect host-related cues to discriminate the physiological condition of the host female at medium/close-range.

The preference of *T. brochymenae* for mated females is strongly related to the transfer of sperm and associated substances to the female during copulation. Evidence of the transfer of sperm and associated nutrients after prolonged copulation has been shown in other pentatomid species (Schrader, 1960; Mitchell and Mau, 1969; Mau and Mitchell, 1978; Kasule, 1986; Koshiyama et al., 1993; Wang and Millar, 1997; Ho and Millar, 2001a; Ho and Millar, 2001b). Moreover, a role of the male secretions as nutrients for the females has been documented in other insect orders such as Blattodea (Mullinus and Keil, 1980), Orthoptera (Gwynne, 1988), Lepidoptera (Bogges and Gilbert, 1979) and Coleoptera (Boucher and Huignard, 1987). Zhan and colleagues hypothesized that *M. histrionica* males determine the mating status of potential partners on the basis of the changes in the cuticular chemistry after copulation (Zhan et al., 2008). In the same way, these variations in the cuticular chemistry may explain how *T. brochymenae* can discriminate the physiological status of the host female.

The compounds that mediated the arrestment response of *T. brochymenae* females seem to be part of the host cuticle. In fact, *T. brochymenae* females showed arrestment responses when exposed to arenas treated by tarsi and scutella dissected from *M. histrionica* mated adults. In all probability the cuticular hydrocarbons present in the wax layer of *M. histrionica* might be involved, as the
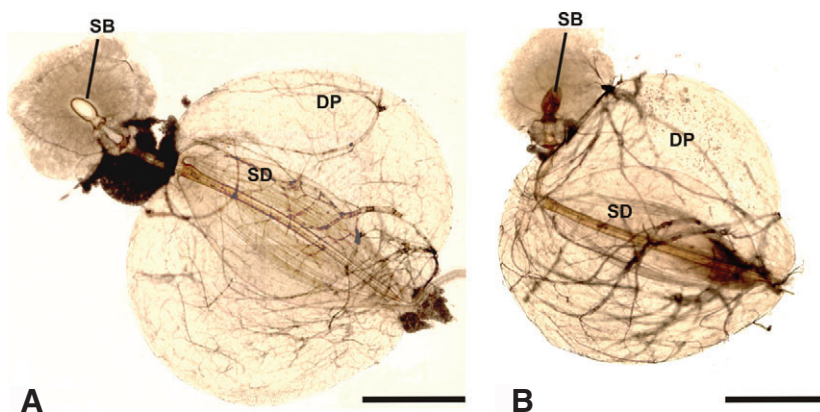

Fig. 3. Spermathecal bulbs (SB) and dilated portion (DP) of spermathecal ducts (SD) dissected from (A) females of *M. histrionica* with interrupted copulation and from (B) mated females that ended the copula. Scale bars: A and B=1 mm.
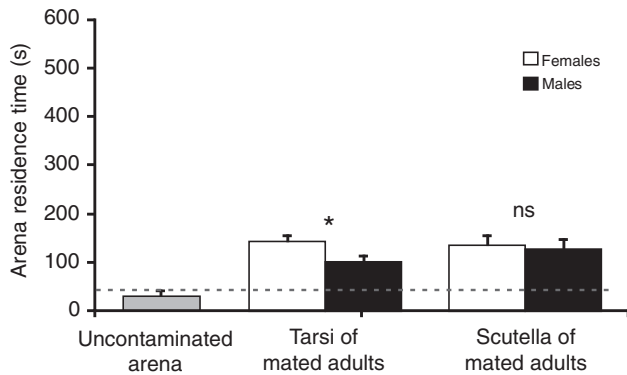
Fig. 4. Residence times (s) of *T. brochymenae* on uncontaminated arenas and on arenas on which dissected body parts (tarsi and scutella) from three mated females and three mated males of *M. histrionica* had been rubbed. Values below the broken line show a lack of arrestment response. Bars indicate means ± s.e.m. (*$P<0.05$; ns, not significant; Student's *t*-test for independent samples).

kairomonal cues are hexane soluble. However, the involvement of other components in the elicitation of wasp response such as mono- and dimethyl-branched alkanes and polar lipids-branched hydrocarbons cannot be excluded. Similar findings have been demonstrated in *N. viridula* as chemicals present on the tarsi and scutella induced motivated searching responses by females of *T. basalis* (Colazza et al., 2007).

The arena residence time of *T. brochymenae* was significantly affected by host gender with a different role played by host body parts. In fact, wasps stayed longer on the arenas treated with legs dissected from mated females than on arenas treated with the analogous body parts dissected from mated males. Therefore, the kairomones that play a role as gender-specific cues seem to be present in the legs of adult *M. histrionica*, and they could be secreted by glands found in the legs of *M. histrionica* (R. Romani and N. Isidoro, personal communication). By contrast, in the case of *N. viridula*, the kairomones, which induce the arrestment response in *T. basalis*, are present in the cuticular lipids distributed all over the body of the insect, even if leg glands similar to those of *M. histrionica* were found in *N. viridula* (Colazza et al., 2007). The mechanism used by *T. brochymenae* to discriminate between
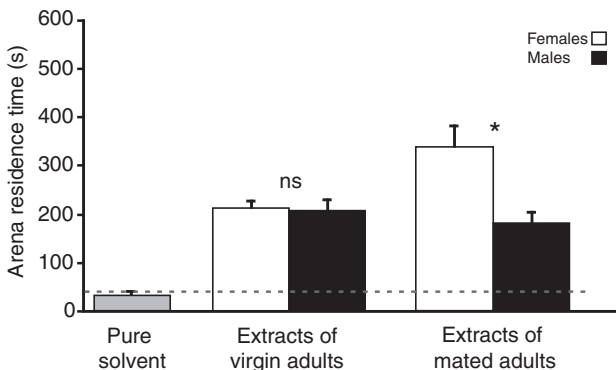

Fig. 5. Residence time (s) of *T. brochymenae* on arenas treated with pure hexane and on arenas treated with hexane extracts of dissected body parts from mated and virgin hosts, applied at the dose of one adult equivalent. Values below the broken line show a lack of arrestment response. Bars indicate means ± s.e.m. (*$P<0.001$; ns, not significant; Student's *t*-test for independent samples).

chemical traces left by mated females that have not yet laid eggs and mated males remains to be investigated.

In conclusion, our results represent an interesting new piece of information regarding the exploitation of indirect host-related cues by egg parasitoids, as they suggest the existence of a new strategy adopted by an egg parasitoid during the host location process.

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