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SHORT COMMUNICATION

Kairomonal effect of walking traces from *Euschistus heros* (Heteroptera: Pentatomidae) on two strains of *Telenomus podisi* (Hymenoptera: Scelionidae)

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Abstract. The semiochemical cues used by geographically isolated strains of the parasitoid, *Telenomus podisi* (Ashmed), to find eggs of the stink bug *Euschistus heros* were investigated. Two strains of *Te. podisi*, maintained on eggs of a South American host (*E. heros*) were studied. One parasitoid strain originated from specimens collected near Brasilia, Brazil (SA strain), and a second strain originated from specimens collected at Beltsville, Maryland (NA strain). Cold tolerance tests of adults from the NA and SA *Te. podisi* strains, analyses of the cuticular hydrocarbons between the two strains, and crossing experiments between strains each indicated consistent differences between the NA and SA strains. Subsequent experiments using *E. heros* showed that SA *Te. podisi* females specifically recognize traces left on the substrate by walking *E. heros* females and then search intensively the area of the 'footprints', apparently looking for an egg mass to parasitize. By contrast, *Te. podisi* females of the NA strain are incapable of recognizing the footprints of *E. heros* females despite the fact that these parasitoids were reared from eggs of *E. heros*. The possibility that the two strains are actually different species is discussed.

Key words. Behaviour, biological control, cold tolerance, cuticular hydrocarbons, egg parasitoid, kairomone, neotropical brown stink bug.

Introduction

Intraspecific variability in biological or behavioural traits of entomophagous parasitoids is important for their efficacy in biological control and has implications for biosystematics (Ruberson *et al*., 1989; Lewis *et al*., 1990).

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The subject of the present investigation, the egg parasitoid *Telenomus podisi* (Ashmed), was shown in laboratory bioassays to recognize its host, *Euschistus heros* (F.), through both chemical and physical cues (Borges *et al*., 1999). In addition, the diel periodicity of the egg parasitoids matches that of their host, *E. heros* (Borges *et al*., 1998). The importance of chemical stimuli for the orientation of female egg parasitoids toward their hosts has been documented for many species (Vinson, 1998). Frequently, long-range attraction is first mediated by host-plant volatiles and/or volatiles emanating from hosts. For example, in the Heteroptera, Leal *et al*. (1995) showed that the egg parasitoid, *Ooencyrtus*

nezarae (Hymenoptera: Encyrtidae), utilizes the attractant pheromone of the bean bug, *Riptortus clavatus* (Alydidae), to locate habitat likely to harbour eggs of this potential host. *Trissolcus basalis* (Hymenoptera: Scelionidae), an egg parasitoid of the stink bug, *Nezara viridula*, homes-in on *(E)*-2-decenal from the defensive metathoracic gland of *N. viridula* (Mattiacci *et al*., 1993). In the case of *Te. podisi*, the male-produced pheromone of *E. heros* is apparently attractive to the wasps at long-distance (Borges *et al*., 1998). Other substances, usually associated directly with the host and often less volatile than long-range cues, are exploited by parasitoids at close-range for host finding and host-recognition (Vinson, 1998; Boo & Yang, 2000). For example, Colazza *et al*. (1999) showed that *Tr. basalis* females remain longer and search more intensively for host eggs in an area where traces have been left on the substrate by walking *N. viridula* adults. Similarly, two strains of the lepidopteran egg parasitoid *Telenomus busseolae* are stimulated to search in areas contaminated by hosts as reported by Colazza & Rosi (2001).

The genus *Telenomus* includes a large number of species and involves a wide range of variation, making a revision at the generic level difficult even for a single biogeographical region. For example, in the Nearctic's domain, there may be more than 200 species (Johnson, 1984). Johnson (1992) considered *Te. podisi*, in particular, to be a highly variable species, especially in the tropics, and even in the temperate region, Ehler (2000) reported two forms of *Te. podisi* based on differences in colouration of the antennal radicle of this species. Studies of Neotropical scelionids using molecular markers reveal considerable variability even among members of the same species (Aljanabi *et al*., 1998).

The variability among species or even among strains of a species is very important for biological control efforts, both classical and augmentative. For example, attempts to use strains of parasitoids maladapted to local climates or with inferior host searching properties often results in biocontrol failures (Parra *et al*., 2002).

In the present study, the response of two geographically isolated strains of *Te. podisi* to the semiochemical cues used in the location of the eggs of the stink bug *E. heros* was investigated. The behavioural responses of two *Te. podisi* strains, one from South America and one from North America, to stimuli emanating from *E. heros* originating from Brazil were compared. In addition, the cuticular hydrocarbon profiles of the two parasitoid strains were examined, and the cold tolerance of adults from each strain was tested.

Materials and methods

Parasitoids

The North American (TpNA) strain of *Te. podisi* originated from individuals that emerged from *Euschistus obscurus* eggs placed in the field at the Beltsville Agricultural Research Center (BARC), Maryland (39°2′N/76°55′W), in 2000 (Aldrich *et al*., 1994; Borges & Aldrich, 1994). The South American (TpSA) strain of *Te. Podisi* originated from a colony started from eggs imported from a colony maintained at EMBRAPA Genetic Resources and Biotechnology laboratory in Brazilia, DF, Brazil since 1996 from specimens field-collected in Brazil, near Brasilia (15°47′S and 47°55W) from parasitized *E. heros* eggs. Both parasitoid strains were maintained in the laboratory on *E. heros* eggs. Fresh egg masses (approximately 200 eggs; $\leq$24 h) were glued to a strip of cardboard with honey and then placed in a 15-mL glass test tube plugged with cotton. Eight females and three males were introduced into each glass tube for mating and for egg parasitism, and held in a growth chamber at 25 $\pm$ 2 °C, 75 $\pm$ 5% RH in a LD 16 : 8 h photoperiod.

Hosts

The BARC *E. heros* colony was started from eggs imported from a colony maintained in the Brasilia laboratory since 1996 from specimens field-collected in Brazil, near Brasilia. The bugs were reared in 5-L plastic containers on sunflower seeds, peanuts, fresh green beans and water, renewed three times a week (Aldrich *et al*., 1994). A paper towel was placed inside against the wall of each container as an oviposition substrate and shelter for the bugs. Egg masses were collected daily and incubated in Petri dishes until emergence of second instars, at which time nymphs were transferred to plastic containers and reared as above.

Female *E. heros* require approximately 2 weeks after ecdysis to the adult stage to become sexually mature (Aldrich *et al*., 1994; Borges & Aldrich, 1994). Ovipositional and preovipositional-mated females were used for the bioassays, but the latter group consisted of females 2–3 days after mating prior to egg laying. Under the laboratory conditions described here, *E. heros* females began laying eggs after the fourth day following mating. Female stink bugs used for all experiments were 2–4 weeks old.

Crossing experiments

Cross mating tests were carried out to check whether or not the two parasitoid strains belonged to the same species. Reciprocal and nonreciprocal (control) crosses between the two strains of *Te. podisi* comprised: (i) introduction of a virgin adult female from one strain into a glass tube containing an adult male of the other strain; (ii) introduction of one *E. heros* egg parasitized by a virgin female *Te. podisi* of one strain into a glass tube containing an egg parasitized by a mated female *Te. podisi* of the other strain; or (iii) introduction of a virgin adult female of one strain into a glass tube containing an adult male of the same strain (control).

Virgin *Te. podisi* pairs, 1–2 days old, of both treatment combinations were confined for approximately 48 h in a test tube to allow copulation. After 48 h, individual females were then placed in a tube with approximately 20 unparasitized *E. heros* eggs. After 24 h, the egg masses were

removed and stored individually until offspring emerged. The crosses were evaluated indirectly by counting the pairs that produced female offspring because this species has haplodiploid sex determination. Each cross was replicated at least 10 times.

From the control crossing experiments of the above-described procedure, data from the development time and sex ratio of the two *Te. podisi* strains were also recorded for further statistical analysis.

Freezing experiments

Adult egg parasitoids; 5–6 days old, of both sexes and from each strain were held at −4 °C for 24, 48, 72, 96, 120 and 144 h. After the prescribed cold treatment, the wasps were warmed to ambient temperature for 24 h, and the number of insects that recovered from the freezing state was recorded. Each time period treatment was replicated at least five times.

Cuticular hydrocarbon analysis

Adult male and female parasitic wasps of the two *Te. podisi* strains (40 adults per sample) were extracted in 2 mL screw-top vials containing 1 mL of *n*-heptanes (high-performance liquid chromatography grade, Aldrich Chemical Co., Milwaukee, WI). After 3 min, the *n*-heptane extract was filtered and collected in 1 mL screw top graduated conical vials, and concentrated to 100 µL under a flow of argon. One microliter of each extract was analysed by GC/MS using a Hewlett-Packard 5890 GC, coupled with Hewlett-Packard 5971 Mass Selective Detector (Hewlett-Packard Inc., CA, USA). Typical reconstructed ion chromatogram (RIC) was obtained by running the sample with DB-5 capillary column (inner diameter 30 m × 0.25 mm, 0.25 µm film-thickness; J & W Scientific Inc., Folsom, CA) in the splitless mode with hydrogen as carrier (60 cm/s linear velocity) programmed from 100 °C for 5 min to 250 °C at 15 °C/min and held for 15 min. Hydrocarbon analyses were replicated a total of three times with sets of independently prepared extracts for each strain.

Open arena behavioural assays

Walking responses and residence times of *Te. podisi* females to residues left by *E. heros* female adults were assayed in an open arena consisting of filter paper (number 1, 24 cm diameter; Whatman, U.K.) where wasps could move in an unconstrained field. In the centre of the filter paper, a circular area 4.25 cm in diameter was either left untreated (control) or was exposed for 1 h to a single *E. heros* female adult constrained under a iron meshed cover (4 cm in diameter, 0.5 cm high, and 0.01 cm mesh), to ensure that the bugs were in continual tarsal contact with the treatment zone of the paper. To produce the 'footprints', an adult female *E. heros* was confined for 1 h under the wire cage placed overtop the test zone. Filter papers contaminated with faeces were not used for bioassays. Following application of the treatments, wasps were gently placed individually in the middle of the circular area. Continuous observation started immediately and stopped when the wasp flew from or walked out of the arena. Wasp responses to stink bug footprints (37 replicates/treatment) were compared with controls in which the test zone was left untreated.

The arena was lit from above and was observed using a video monitor (Sony SSM-14 N5E) connected with a monochrome CCD camera (Sony SPT M324CE) fitted with a 12.5–75-mm/F1.8 zoom lens (Alarmax Distributor Inc., Beltsville, USA). A video frame grabber (PC-Studio PCTV Pinnacle System-http://www.pinnaclesys.com) digitized analogue video signals from the camera, and data were processed using 'Xbug' Software (Colazza *et al*., 1999). We computed the arena residence time(s) (i.e. the time from when a wasp first entered the arena until it flew from or walked out of the arena). To quantify wasp's kinetic reactions (i.e. their adjustment in direction of the movement comparative to the distance moved), a novel index, named tortuosity index, as a measure for the wasp's linearity of movement, was applied. From the coordinates of the insect (sample rate = 15 images/s), the tortuosity index was computed as:

$$\text{Tortusity index} = 1 - mp/tl$$

where *mp* is the projection of the track in general straight line of the plan, and *tl* is the total length of the track. The value can range from 0 to 1, with 0 indicating a completely linear tracking and 1 the maximum of tortuosity.

Freshly emerged *Te. podisi* females of both strains were kept for approximately 24 h with males, then they were individually isolated and supplied with a drop of pure honey. The females used in this experiment were 2–3 days old, and had not previously oviposited, nor had they been in contact with the host or its body, and they were used only once during the bioassays. All experiments were carried out from 09.00 h to 12.00 h. The temperature in the bioassay room was kept at approximately 24 °C.

Statistical analysis

Data were tested for normality (Kolmogorov–Smirnov test) and, because there was no significant deviation from normal distribution, the variable values were then analysed with parametric tests (*t*-test). All the data were analysed using the statistical software package, Statistica 5.1 (StatSoft, Inc. 1997). Fisher's exact probability test (StatSoft, 1997) was used to analyse the reciprocal and nonreciprocal (control) crosses between two strains of *Te. podisi*. The numbers of insects that recovered from the freezing experiments were expressed as percentages.

Results and discussion

Crossing experiments

The results showed that all crosses produced only males, indicating that successful mating between the Nearctic's and

Neotropical *Te. podisi* strains did not occur. Moreover, the control crosses resulted in successful mating, and the sex ratios obtained from the control experiments were significantly different from each other (Table 1).

Freezing experiments

There was a large difference in cold tolerance individuals from the Nearctic's strain and wasps from the Neotropical strain (Fig. 1). The NA *Te. podisi* are able to recover after freezing periods of more than 120 h (> 80% survival after 5 days at −4 °C), a trait which facilitates handling of this potential biological control agent.

Cuticular hydrocarbons analysis

Although there was little sex-specific difference in the cuticular hydrocarbon profiles within either *Te. podisi* strain, between strains the profiles were vastly different (Fig. 2). No obvious variation (qualitative/quantitative) was found among the three sets of cuticular extracts that were analysed for each strain. Because the prime goal of the cuticular hydrocarbon analysis was to distinguish two *Te. podisi* strains by hydrocarbon profile, we did not pursue chemical identification of individual compounds.

Open arena behavioural assays

After introduction into the control arena, females of both *Te. podisi* strains usually searched the arena in an apparently random pattern. The search patterns of the two strains in the absence of the host stimuli were not significantly different for both the parameters measured; arena resident time and tortuosity (Fig. 3A). By contrast, after introduction into a treated arena, TpSA females exhibited a distinctive searching behaviour in the vicinity of traces left by the *E. heros* female. Compared with the TpNA females, TpSA females spent significantly more time in the arena on which an *E. heros* ovipositional female walked than did TpNA females (Fig. 3B). The TpSA females turned back into the treated area when the edge of the zone was

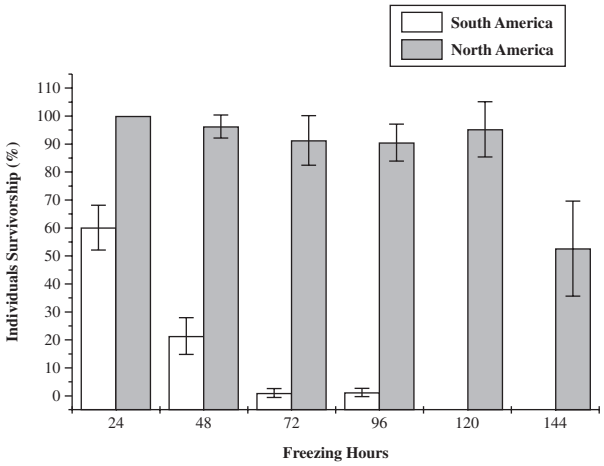

Fig. 1. Sub-freezing temperatures (−4 °C) cold tolerance (% ± SE) of adult wasps from North (TpNA) and South (TpSA) American strains of *Telenomus podisi* for different time periods; $n = 180$ (average number of individuals) and $n = 10$, for the South American (TpSA) strain of *Te. Podisi*; $n = 80$ (average number of individuals) and $n = 6$, for the North American (TpNA) strain of *Te. Podisi*.

detected, changing their kinetic reaction as shown by the difference in the tortuosity index, which was significantly greater than that for TpNA females exposed to the same treatment (Fig. 3B).

Earlier research with *Te. podisi* in Brazil indicated that the South American strain of this egg parasitoid arrives in the vicinity of reproductive adults of *Euschistus heros* by orienting toward the allomone and/or pheromone compounds of the potential host (Borges *et al.*, 1998). Here, we have shown that SA *Te. podisi* females specifically recognize traces left on the substrate by walking *E. heros* females and then intensively search the area of the 'footprints', apparently looking for an egg mass to parasitize. By contrast, the NA *Te. podisi* females are incapable of recognizing the footprints of *E. heros* females, despite the fact that these parasitoids were reared from eggs of *E. heros*. The influence of the host treated arena was significantly

Table 1. Reciprocal and nonreciprocal (control) crosses between two strains of *Telenomus podisi*.

		Progeny					
Cross mating	Total pair of cross mating	♂	♀	Total	Average number of offspring produced by each female	Sex ratio (♀/♀ + ♂)	Mean sex ratio
♀ SA × ♂ NA	11	157	0	157	14.3 ± 3.0	0	0
♀ NA × ♂ SA	11	158	0	158	14.4 ± 2.9	0	0
Egg (virgin ♀ SA) × Egg (mated ♀ NA)	10	146	0	146	14.6 ± 4.4	0	0
Egg (virgin ♀ NA) × Egg (mated ♀ SA)	10	138	0	138	13.8 ± 5.1	0	0
♀ SA × ♂ SA	10	30	97	127	12.7 ± 4.2	0.7*	0.12 ± 0.002a*
♀ NA × ♂ NA	10	67	67	134	13.4 ± 6.6	0.5**	0.21 ± 0.003b**

*One female produced only male progeny.

**Four females produced only male progeny. Results are given as means ± SD. Values in a column followed by the same letters are not significantly different at the 5% level (Fisher's exact test). Sex ratios (expressed as male percentage) were arcsine-transformed before analysis and subsequently analysed by Fisher's exact probability test (StatSoft, 1997).

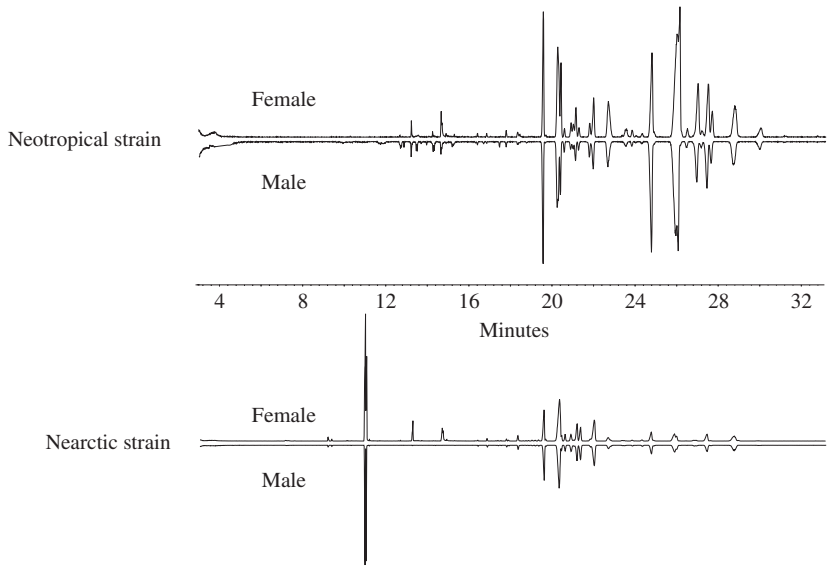

Fig. 2. Typical reconstructed ion chromatogram (RIC) obtained from cuticular hydrocarbon profiles showing the difference between parasitoid strains; the largest peak was automatically normalized to 100%, $n = 3$ (with sets of independently prepared extracts for each strain). (A) Chromatogram obtained from the South American (TpSA) strain of *Telenomus podisi*. (B) Chromatogram obtained from the North American (TpNA) strain of *Te. podisi*.

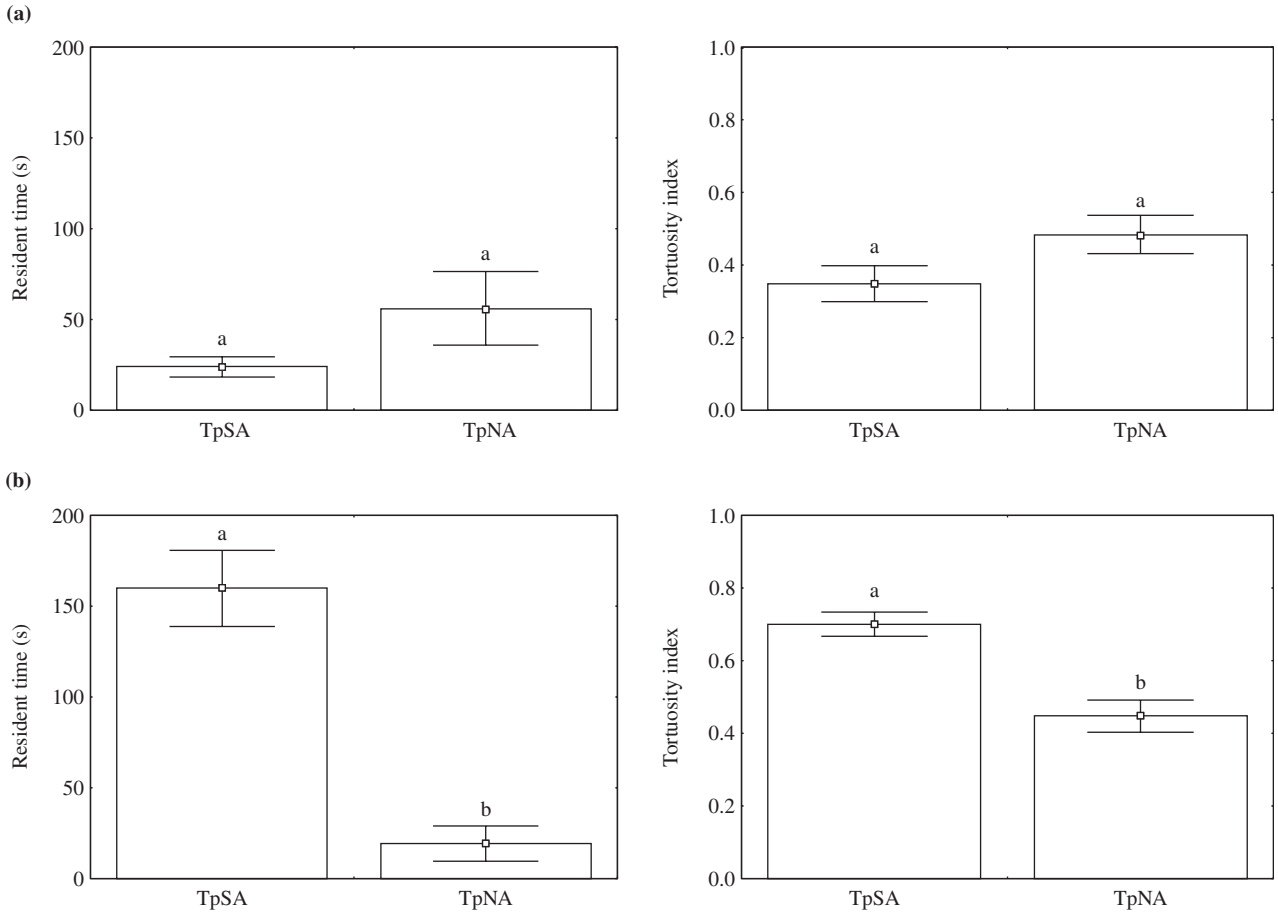

Fig. 3. Comparison of resident time and tortuosity index among the two different strains of *Telenomus podisi* when in contact with an untreated open arena (A), or when in contact with an open arena treated with the traces left by a mated female of *Euschistus heros* (B) (mean ± SE). Bars with the same letters are not significantly different at $P < 0.01$ (*t*-test) ($n = 37$). TpSA = South American strain of *Te. podisi*; TpNA = North American strain of *Te. podisi*.

different when the two strains were compared. The Neotropical *Te. podisi* strain responded positively to the treated arena by displaying the characteristic searching behaviour of scelionid wasps, as described by Colazza *et al.* (1999) for *Tr. basalis* searching for traces left by its host, *N. viridula*.

Cuticular hydrocarbons fulfil a multitude of roles and uses, including colony and nest mate recognition in social insects, discrimination of species, definition of larval karyotypes and, for taxonomic purposes, distinction between lineages (Anyanwu *et al.*, 2001; Lahav *et al.*, 2001; Akino *et al.*, 2002; Nelson *et al.*, 2002; Page *et al.*, 2002; Quelle & Strassmann, 2002; Ruther *et al.*, 2002). Cuticular hydrocarbons also frequently play a role in mate discrimination in insects (Ishii *et al.*, 2002). Consistent differences were found in cuticular hydrocarbons of the two closely related strains of *Te. Podisi* used here. Although there was little sex-specific difference within either strain, there was a great deal of divergence in the chemical profiles between the *Te. podisi* from North vs. South America.

Examination of a second physiological trait (cold tolerance) also revealed a dramatic discrepancy in the abilities of wasps from the two strains to withstand freezing. North American *Te. podisi* females are extremely cold tolerant ($> 80\%$ survival after 5 days at $-4\,^{\circ}C$). Although this trait would facilitate tremendously the handling of this potential biological control agent, mass rearing and release of this strain in Brazil, or any other country where *E. heros* species are pests, is not worthwhile because females of NA strain are unable to track *E. heros*.

In summary, attempts to hybridize the North and South American strains of *Te. podisi*, in an effort to create a strain whose females are both cold-tolerant and efficient searchers, have repeatedly failed. This interstrain sterility, plus the major chemical, physiological and behavioural differences demonstrated here for the two strains, raises the distinct possibility or even likelihood that the so-called strains are actually separate species. Nevertheless, efforts are continuing to identify the footprint compounds from *E. heros* females that are recognized by South American *Te. podisi* female wasps, and to select a strain of the parasitoid combining cold tolerance and efficient host searching.

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