Phoresy in the field: natural occurrence of *Trichogramma* egg parasitoids on butterflies and moths

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Abstract Phoretic insects utilize other animals to disperse to new environments. We recently discovered how egg parasitoids use an exciting phoretic strategy to reach egg-laying sites of their butterfly hosts. In the laboratory, female *Trichogramma* wasps detect and mount mated female cabbage white butterflies that emit an anti-aphrodisiac pheromone. Hardly any information exists about the natural occurrence of phoresy in wasps of this genus. Therefore, we monitored the presence of phoretic *Trichogramma* wasps on lepidopteran hosts in the field. Only female wasps were found at low prevalence on six lepidopteran species. Wasps were mostly found on female hosts and mainly on abundant solitary host species. This is the first report of phoretic *Trichogramma* wasps on butterflies in nature. We suggest that phoresy is only one of several strategies used by these polyphagous egg parasitoids. The evolution of phoresy is discussed in relation to the nutritional ecology of egg parasitoids.

Keywords Hitchhiking · Anti-aphrodisiac · Oviposition-induced plant cues · Lepidoptera · Gregarious · Solitary

Introduction

Small animals with low mobility often seek out vehicles to migrate to new environments, for further development or reproduction (Binns 1982; Clausen 1976; Southwood 1962). Transportation on the bodies of other organisms, for purposes other than direct parasitism, is termed phoresy (see Clausen 1976 and references therein). Phoresy is commonly observed in arthropods like mites (Binns 1982). In parasitoid wasps it is almost exclusively restricted to species that develop in the smallest life stage of an insect: the egg (Clausen 1976; Godfray 1994; Vinson 1998). Because insect eggs are often inconspicuous, such egg parasitoids strongly rely on indirect cues emitted by adult hosts to obtain access to their eggs (Colazza et al. 2010; Fatouros et al. 2008b). In addition, egg parasitoids are generally assumed to be incapable of overcoming long distances by directed flight (Noldus 1989; Smith 1996; Suverkropp et al. 2009). This puts a strong selective pressure on these wasps to search for transport vehicles to get near their target host stage.

Phoretic relationships in egg parasitoids are frequently observed in the hymenopteran family Scelionidae, in some species of Trichogrammatidae, Eulophidae and single species of Encyrtidae,

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Eupelmidae and Torymidae [see Table 1 in electronic supplementary material, an update of the overview by Clausen (1976)]. In total, about 35 egg parasitoid species are reported to be phoretic. Thus, from the nearly 4,000 described egg parasitoid species belonging to the two families, Scelionidae and Trichogrammatidae (Austin et al. 2005; Querino et al. 2010), only about 1% are known to be phoretic. The hosts they use for transport include moths, grasshoppers, butterflies, pentatomid bugs, mantids, beetles, a dragonfly, an ant, and a fly (Table 1 in supplementary material). Once located, the wasps attach to their hosts either on the wings (of moths, butterflies and mantis), the abdomen (grasshoppers) or sometimes even on the hosts' antennae (of a bug, fly or ant) (Table 1 in supplementary material).

Some phoretic relationships can be obligate like between the European mantis (*Mantis religiosa* L.) and scelionid wasps of the genus *Mantibaria*, which parasitize the mantis' egg masses (Bin 1985; Couturier 1941). Here, the female wasp removes her wings after having mounted the female mantis, and hitches a ride to the oviposition site. The wingless wasp is then able to enter the frothy coating of freshly laid mantis eggs. Afterwards the parasitoid often returns to the same female mantis to parasitize successive egg masses. In other cases of phoresy, the adult host stage can strongly attract parasitoids: up to 250 females of the trichogrammatid *Xenofens* sp. were found on a female *Caligo eurilochus* (Cramer) (Lepidoptera: Nymphalidae) butterfly. This phoretic association is highly advantageous for the trichogrammatid wasps: they reach a mean parasitism rate of 78% of the butterfly eggs (Malo 1961).

To localize their vehicles, phoretic arthropods use cues of host activity, including auditory and tactile cues (Harbison et al. 2009; Owen and Mullens 2004) and infochemicals emitted by the host (Arakaki et al. 1996; Bruni et al. 2000; Soroker et al. 2003). In some cases, vehicles are even lured by the phorents by sexual deception (Saul-Gershenz and Millar 2006). Only few studies have investigated chemical cues that are used by phoretic egg parasitoids to locate their transporting host (Table 1 in supplementary material). Females of the egg parasitoid *Telenomus euproctidis* Wilcox (Hymenoptera: Scelionidae) are attracted by the sex pheromone of virgin Tussock moths (*Euproctis pseudoconspersa*) and hide in the anal tuft until the female moth started laying her egg clutch (Arakaki et al. 1995, 1996). Another

scelionid wasp, *Telenomus calvus* Johnson, utilizes an aggregation pheromone emitted by male spined soldier bugs to find its host. When a male bug mates, the wasp transfers onto the female, which is possibly recognized by female-specific volatiles that are released from small glands underneath the wings (Aldrich 1995; Aldrich et al. 1984; Bruni et al. 2000; Buschman and Whitcomb 1980).

We recently reported about a combination of chemical espionage and phoresy in egg parasitoids of the genus *Trichogramma*. These polyphagous wasps parasitize eggs of a wide range of Lepidoptera, and are therefore massively used in biological control worldwide (Parra 2010; van Lenteren 2000) with currently 15 species commercially available on the world market (van Lenteren 2012). Two *Trichogramma* species utilize anti-aphrodisiac pheromones emitted by mated female hosts: the gregarious Large Cabbage White *Pieris brassicae* L. (Lepidoptera: Pieridae), that deposits egg clutches, and the solitary Small Cabbage White *P. rapae* L., that lays only a single egg at a time (Fatouros et al. 2005b; Huigens et al. 2009, 2010, 2011). Anti-aphrodisiacs are transferred from males to females during mating to enforce female monogamy (Andersson et al. 2000, 2003). Mated females were most attractive to the wasps (Fatouros et al. 2005b; Huigens et al. 2010). Synthetic anti-aphrodisiac compounds arrested inexperienced *T. brassicae* when applied onto virgin females, and stimulated the wasps to mount their hosts (Fatouros et al. 2005b; Huigens et al. 2010, 2011). After hitching a ride on a mated female *Pieris* butterfly to a host plant, e.g. Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*), a *T. brassicae* wasp descends from the butterfly and parasitizes her freshly laid eggs. In contrast, inexperienced wasps of the closely related *T. evanescens* have to associatively learn to respond to anti-aphrodisiacs of cabbage white butterflies (Huigens et al. 2009, 2010). These behavioural studies were all performed under laboratory and greenhouse conditions. Very limited information is available about the prevalence of phoresy among *Trichogramma* species in nature. To the best of our knowledge, there is only one published report of *Trichogramma* females found on the moth *Dendrolimus sibericus albolineatus* Mats. (Lepidoptera: Lasiocampidae) in Russia (Tabata and Tamanuki 1940).

For egg parasitoids with numerous eggs available and a short lifespan, such as wasps of the genus

Trichogramma, phoresy should be especially adaptive on gregarious hosts and on hosts that lay large single eggs per oviposition bout. In that case one successful phoretic event would give a female wasp the opportunity to deposit many of her eggs (Fatouros et al. 2005b). The aim of this study was to investigate the prevalence of phoresy in *Trichogramma* wasps in natural and cultivated habitats in the Netherlands and to test whether phoretic wasps are more frequently found on gregarious Lepidoptera than on solitary ones. So far, there are seven described *Trichogramma* species occurring in the Netherlands: *T. evanescens* Westwood, *T. brassicae* (Synonymy: *T. maidis*) Bezdenko, *T. aurosum* Sugonjaev and Sorokina, *T. semblidis* (Aurivillius), *T. cacoeciae* Marchal, *T. embryophagum* (Hartig), and *T. dendrolimi* Matsuura (Polaszek 2010). Here, we collected adults of a wide range of potential solitary and gregarious lepidopteran host species in the field, and examined them for the presence of phoretic *Trichogramma* wasps. Moreover, we updated the latest reports on phoretic relationships in egg parasitoids since Clausen (1976).

Materials and methods

Sampling sites

Adult butterflies were collected from 2003 to 2009 in natural and cultivated field sites in the Netherlands that were mostly located around the city of Wageningen (51°57'N–51°58'N, 5°38'E–5°39'E). Moths were only collected in 2007. The main field sites varied between (1) a small garden/meadow with flowering plants (2), a cultivated cabbage field with *Brassica oleracea* plots, (3) a botanical garden, (4) a meadow with flowering plants, (5) black mustard (*Brassica nigra* L.) patches surrounded by meadows near the river Rhine, and (6) a meadow with flowering plants close to that same river. Other occasional sampling sites were located close to the cities of Nijmegen (51°48'N–51°50'N, 5°54'E–5°55'E), Ede (52°35'N, 5°39'E) and Alphen (51°27'N–51°30'N, 4°56'E–4°59'E).

Butterfly and moth catching

Butterflies were collected with a net (Diameter 30 cm, Vermandel, The Netherlands) that contained a plastic

vial (height 7 cm, diameter 7 cm) at the bottom to avoid loosing phoretic wasps while catching the butterfly as much as possible (see Fig. 1). After the butterfly entered the vial it was closed with a lid and replaced by a new vial. To prevent accidental catching of non-phoretic wasps, butterflies were always caught in flight. In case a butterfly was present on a plant/flower, we waited until it took off to make sure no plant material ended up in the butterfly net with vial inside. Moreover, we performed 138 control sweeps through the vegetation at the six main sampling sites without catching butterflies. This did not yield any *Trichogramma* wasps. Moths were collected during night. At night an High Performance Lamp light powered by a generator was used to attract moths to a big white cloth (6 m²). Moths that landed on the cloth were caught using the same plastic vials as those inside the butterfly nets. After collection, vials with butterflies and moths were placed in a refrigerator to reduce mobility and thereafter the lepidopteran species and sex was determined. Furthermore, butterfly/moth and vial were examined under a stereomicroscope for the presence of *Trichogramma* wasps. Wasps were put in small vials containing 95% ethanol for molecular species identification based on the ribosomal ITS-2 gene (for more details see Gonçalves et al. 2006; Huigens et al. 2004; Stouthamer et al. 1999).

Wasp species identification

DNA extraction

The wasps that were stored in ethanol were dried on filter paper. Afterwards, they were individually crushed in a 0.5 ml Eppendorf tube with a closed pasteur pipet. Then, 50 µl of Chelex solution (5%) and 4 µl of proteinase K (20 mg ml⁻¹) were added. The samples were incubated overnight at 56°C, followed by 10 min at 95°C.

PCR amplification

For every sample a PCR reaction was performed. PCR reactions were performed in a 25 µl volume consisting of 18.43 µl of distilled water, 2.5 µl 10× PCR reaction buffer (HT Biotechnologies Ltd., Cambridge, UK), 2.5 µl DNA template, 0.5 µl dNTP (10 mM), 0.5 µl forward primer, 0.5 µl reverse primer and 0.07 µl Taq polymerase (5 U µl⁻¹) (HT Biotechnologies Ltd.,

Fig. 1 Percentage of butterflies carrying a phoretic *Trichogramma* wasp in our field survey in The Netherlands. Number above the columns represent the number of collected individuals per butterfly species and gender. Sampling was done with an adapted net containing a vial at the bottom. Pictures of butterflies are not in natural proportion to each other

Cambridge, UK) within a 0.2 ml Eppendorf tube. The primers used to amplify the ITS-2 region were:

5'-TGTGAACTGCAGGACACATG-3' (forward)
and
5'-GTCTTGCTGCTCTGAG-3' (reverse).

The PCR cycling program was 3 min at 94°C, 33 cycles of 40 s at 94°C, 45 s at 53°C and 45 s at 72°C, followed by 10 min at 72°C after the last cycle. PCR products were run on a 1.5% agarose gel and stained with ethidium bromide.

Cloning and sequencing

After conducting a PCR reaction and running the samples on an electrophoresis gel, the ITS-2 products were excised from the gel using the MinElute Gel Extraction Kit (QIAGEN GmbH, Hilden, Germany) for DNA fragment purification. The PCR fragment was ligated to a pGEM-T vector (Promega, Madison, WI, USA) and transformed into *Escherichia coli* ×12 cells (Stratagene, La Jolla, CA, USA). Correct insertion of the ITS-2 fragments was confirmed by PCR. To purify the plasmid, a GeneElute Plasmid Miniprep Kit (Sigma-Aldrich Chemie, Steinheim, Germany) was used. ITS-2 fragments were sequenced using an

Applied Biosystems automatic sequencer. Finally, the ITS-2 sequences were aligned and matched against sequences present in GenBank and those present in the large database of Prof. Dr. R. Stouthamer (Department of Entomology, University of California Riverside, USA).

Results

In total, 24 butterfly species were collected and examined for the presence of *Trichogramma* wasps (Supplementary Table 2). From the 1,472 collected butterflies, only ten individual female wasps were found on five different butterfly species, three Pierid and two nymphalid butterflies: the solitary Small Cabbage White *Pieris rapae* (three wasps), the gregarious Large Cabbage White *P. brassicae* (one wasp), the solitary Green-veined White *P. napi* L. (four wasps), the solitary Painted Lady *Vanessa cardui* L. (Lepidoptera: Nymphalidae) (one wasp) and the solitary Meadow Brown *Maniola jurtina* L. (Lepidoptera: Satyridae) (one wasp) (Fig. 1). Male wasps were not found. Butterflies never carried more than one phoretic female wasp. Interestingly, most wasps (nine out of ten) were found on solitary butterflies (Fig. 1).

Fifteen out of the 24 collected butterfly species have a solitary life style. Many more individuals of the solitary species were collected ($N = 1,205$) than from the nine gregarious butterfly species ($N = 267$) (Supplementary Table 3). Most of the wasps (seven out of ten) were found on female butterflies (Fig. 1). We identified two wasp species: six *T. evanescens* were found on three *Pieris* species (one on a *P. brassicae* female, one on a *P. rapae* female, one on a *P. rapae* male, one on a *P. napi* female, and one on a *P. napi* male), whereas *T. brassicae* was found on two *P. napi* females and on one *M. jurtina* female (Fig. 1; supplementary Table 2). Unfortunately, the female wasp that was found on *V. cardui* could not be identified to species level. Our PCR method yielded an ITS-2 sequence that was related, but not identical, to any of the known ITS-2 sequences of trichogrammatid wasps.

From the 777 moths collected belonging to 111 species (Supplementary Table 4), only one night-active female *Xestia c-nigrum* L. (Lepidoptera: Noctuidae) (out of 66 moths collected of that species) carried a female wasp, which was identified as *T. brassicae* (Supplementary Table 2). Internal Transcribed Spacer-2 gene sequences of all phoretic wasps were submitted to GenBank (accession numbers JN315370–JN315380).

Discussion

Our field sampling revealed that phoretic *Trichogramma* wasps occur at low prevalence on adults of various butterfly and moth species in nature. Females of the two species (*T. evanescens* and *T. brassicae*) that display phoretic behaviour on *Pieris brassicae* and *P. rapae* in laboratory studies (Fatouros et al. 2005b, 2007; Huigens et al. 2009, 2010) were also found on butterflies and moths in the field. *Trichogramma evanescens* was only found on three *Pieris* species and is known to be an abundant egg parasitoid of those species in different habitats in The Netherlands (Huigens and Fatouros, unpublished data; van Rijswijk 2000). Phoretic wasps are mostly collected on female butterflies but sometimes also occur on males. In the latter case, those wasps may be inexperienced. We have shown earlier that inexperienced *T. evanescens* females do not discriminate between climbing onto female and male butterflies of *P. brassicae* and *P. rapae* but do prefer to mount

butterflies over non-host insects, such as desert locusts (Huigens et al. 2009). Only after a successful hitchhiking experience on a mated (and thus egg-laying) female butterfly, leading to an oviposition into a freshly laid butterfly egg, these wasps prefer to climb onto mated females over virgin females and males (Fatouros et al. 2007; Huigens et al. 2009, 2010). It might also be that wasps occasionally hitchhike with males to transfer onto females during mating, which has been frequently observed in phoretic egg parasitoids of bugs (Bruni et al. 2000; Kohno 2002; Malo 1961).

To transfer onto or between vehicles, the phoretic passengers need to find sites likely to meet their hosts. Flowers were shown to serve as transmission locations for crab spiders, mites and even pathogens to transfer onto flower visitors, e.g. bumble bees and butterflies during their nectar feeding (Durrer and Schmid-Hempel 1994; Morse 1986; Schwarz and Huck 1997; Treat 1969). As for most parasitoid species, sugars obtained from floral nectar are an essential energy source to sustain many physiological processes. Flower location in these parasitoids is mediated by both olfactory and visual stimuli (Romeis et al. 2005). Thus, since both the butterfly host and *Trichogramma* use floral nectar, flowers may be used for horizontal transfer. On the practical side, the butterfly is stationary during nectar feeding, making it easier for the parasitoid to mount.

Although members of the parasitoid family Trichogrammatidae are well-known to parasitize eggs of many Lepidoptera (Querino et al. 2010), including some of those collected in our survey (Supplementary Table 2), the low prevalence of phoretic wasps in our field study suggests *Trichogramma* species not to heavily rely on transportation on adult hosts to find host eggs. The low prevalence of phoretic *Trichogramma* wasps in our study could have several reasons:

- (1) *Host age and quality*: Orr et al. (1986) reported a number of behavioural and biological adaptations of the phoretic scelionid *Telenomus calvus*, like a narrow range of host egg ages suitable for parasitism. Because of the rather weak ovipositor typical for scelionids (reviewed by Quicke 1997), *T. calvus* is restricted to soft and young host eggs less than 12 h old. Being able to attack only young hosts means that parasitoids need to

seek for them by using a so-called ambush approach, which is ‘to be there before the host egg is oviposited’ (Vinson 1985, 2010). A phoretic habit is one possible approach to accomplish this and could explain why it has evolved frequently among scelionids. In contrast, *Trichogramma* wasps are able to parasitize older host eggs until the head capsule of the caterpillar has sclerotized (reviewed by Pak 1986). They kill the embryo, feed on dead tissues and then can take time to develop (Vinson 2010). *Trichogramma brassicae* wasps were shown to successfully parasitize up to four-day-old *P. brassicae* eggs (Fatouros et al. 2005a).

- (2) *Egg load*: another life history trait related to phoretic behaviour is the relatively small number of eggs produced, for example, by *T. calvus* females (Orr et al. 1986). Usually, all eggs can be deposited in the first encountered host egg clutch. Compared to most scelionids, trichogrammatids are facultative gregarious parasitoids and produce a higher number of, sometimes more than 100 eggs per female (Jervis et al. 2008).
- (3) *Dietary specialization*: *Trichogramma* wasps are very polyphagous (Smith 1996). *Trichogramma evanescens*, for example, parasitizes more than 65 different species (Hase 1925), whereas scelionids appear to be more specialized (Vinson 2010). Polyphagy increases the chance to encounter hosts and would decrease the adaptive value of using specific host-related cues (Vet and Dicke 1992; Vet et al. 1995). A phoretic relationship is likely to require specificity. The ability of the passenger to detect host-related cues depends on the degree of specialization of the interaction (Vet and Dicke 1992). Inexperienced *T. evanescens* can discriminate between a butterfly host and a locust but learn odors of mated *Pieris* females labeled with an anti-aphrodisiac pheromone (Huigens et al. 2009, 2010). This ability of learning to respond to adult host cues is probably related to their relatively wide dietary breadth (Hase 1925). Yet, some *Trichogramma* species/populations may be rather host specific. For example, *T. kaykai* mainly parasitizes eggs of the Mormon Metalmark butterfly (*Apodemia mormo deserti*) in the Mojave Desert of the American Southwest (Huigens et al. 2000; Pinto et al. 1997;

Stouthamer et al. 2001), which lays its eggs on patchily distributed desert trumpet plants (*Eriogonum inflatum*). In such an extreme habitat, it is likely that *T. kaykai* shows to some extent a phoretic life-style.

- (4) *Use of different foraging strategies*: to locate host eggs, most egg parasitoids strongly rely on the adult host stage by using aggregation or (anti-)sex pheromones and other host-related chemical cues released before or during oviposition (Colazza et al. 2010; Fatouros et al. 2008b; Powell 1999; Vinson 2010). However, more evidence accumulates that egg parasitoids also rely on plant cues induced by herbivore egg deposition (Colazza et al. 2010; Hilker and Meiners 2006, 2010). We recently discovered that eggs of *P. brassicae* and *P. rapae* induce chemical changes in Brussels sprouts plants (*B. oleracea* var. *gemmifera*) that arrest *Trichogramma* wasps three days after egg deposition in the close vicinity (Fatouros et al. 2005a, 2009; Pashalidou et al. 2010). Interestingly, these plant cues are induced by the same anti-aphrodisiac compounds that attract *Trichogramma* wasps directly to mated *Pieris* females (Fatouros et al. 2008a, 2009). The black mustard *B. nigra* even releases volatile cues induced by *Pieris* eggs that can attract the wasps from a longer distance (N.E. Fatouros, unpublished data). It is unlikely that oviposition-induced plant cues are used by egg parasitoids that seek for fresh host eggs (i.e. scelionids) because of the delay of the plants’ response to oviposition (Vinson 2010).
- (5) *Sampling method*: despite the use of special butterfly nets (Fig. 1), our methodology may be not highly efficient. We may still loose many phoretic wasps while catching butterflies. Preliminary greenhouse tests showed that increasing the size of the vial from 7 cm (used in the present study) to 15 cm in diameter increased the chance of recapturing a phoretic female *T. brassicae* wasp from a female *P. brassicae* butterfly inside a net from only 10 to 70%, respectively, simply because use of a larger vial inside increases the chance that a butterfly with wasp immediately flies into the vial (Huigens, unpublished data). In case we found wasps, it was almost exclusively on Lepidopteran species of which many individuals were collected (see supplementary

Tables 3 and 4 for a complete overview). A small sample size may also explain why some of the Lepidoptera that are known to be hosts for *Trichogramma* spp. did not yield any phoretic wasps (Supplementary Table 2). Our field study at least highlights that large sample sizes may be required to determine if phoresy occurs in certain species of egg parasitoid wasps, certainly when the prevalence is low and the methodology is not highly efficient. Phoresy might thus be much more widespread in egg parasitoids than known so far (Table 1 in supplementary material).

So far, most hosts known to be exploited by phoretic egg parasitoids have a gregarious life style (>90%) (Supplementary Table 1). Phoresy provides the wasps access to large numbers of eggs oviposited in widely dispersed locations (e.g. grasshoppers) or to eggs sealed in ‘containers’ (i.e. ootheca) such as occurs with mantids (Clausen 1976). Yet, most phoretic *Trichogramma* wasps were found on solitary hosts, laying single eggs. The reason for this might be that most solitary butterfly species with a phoretic wasp were caught in high numbers of more than hundred individuals, which was not the case for the gregarious species. The solitary butterfly species carrying wasps (*P. napi*, *P. rapae*, *V. cardui* and *M. jurtina*) are very abundant in the Netherlands (Bos et al. 2006). Accordingly, the wasps face a trade-off between less frequent gregarious species *versus* abundant solitary ones. Additionally, not only the number of host eggs but also host egg size plays a role in how many offspring a *Trichogramma* wasp can produce. The larger the host eggs, the more wasps will generally develop inside it (Godfray 1994; Schmidt and Smith 1987). Unfortunately, we were unable to evaluate the number of offspring produced per host egg (clutch) of each collected species. We can at least conclude that female *Trichogramma* wasps need to find many eggs of the solitary butterfly and moth species on which we found wasps, to be able to get rid of all the eggs that they produce during their lifespan (Supplementary Table 2).

We do not expect *Trichogramma* wasps to frequently hitchhike with night-active lepidopteran hosts. *Trichogramma* wasps are diurnal wasps that generally become active at temperatures above 12°C (Pak and van Heiningen 1985; Suverkropp et al.

2001). The low prevalence of wasps in our night catches (0.13%) is reflecting this. Yet, two other observations of *Trichogramma* wasps found on a *Xestia baja* and a *Phlogophora meticulosa* moth (Supplementary Table 2; J. Voogd, personal communication) show that certain wasps have found a way to overcome this problem.

To the best of our knowledge, our study presents the first extensive field survey of phoretic *Trichogramma* wasps on adult lepidopterans. Even though *Trichogramma* wasps can reach rather high parasitism rates in the field, e.g. of *Pieris* butterfly eggs in the Netherlands (Fatouros and Huigens, unpublished data; van Heiningen et al. 1985), our data suggest that only a small portion of *Trichogramma* wasps use adult hosts as transport vehicles to oviposition sites. Besides host specificity, patchy or extreme habitats, like deserts, might select for the evolution of a phoretic relationship (Houck and Oconnor 1991; Krishnan et al. 2010; Vinson 1985). Extensive field studies correlating egg parasitism by *Trichogramma* species to the prevalence of phoretic wasps are needed to confirm this hypothesis. From an applied perspective, knowledge about the prevalence of phoretic behavior in a given parasitoid wasp species can be important to increase the efficiency of its use in biological control programs. In case an egg parasitoid heavily relies on phoresy to find freshly laid host eggs, such as *Telenomus* spp., it could be essential to release the wasps before most adult pests start laying their eggs. Our study suggests that this does not apply so much to *Trichogramma* wasps as biological control agents against lepidopteran pests because they can also parasitize “older” host eggs and do not seem to heavily rely on adults as transporting hosts.

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