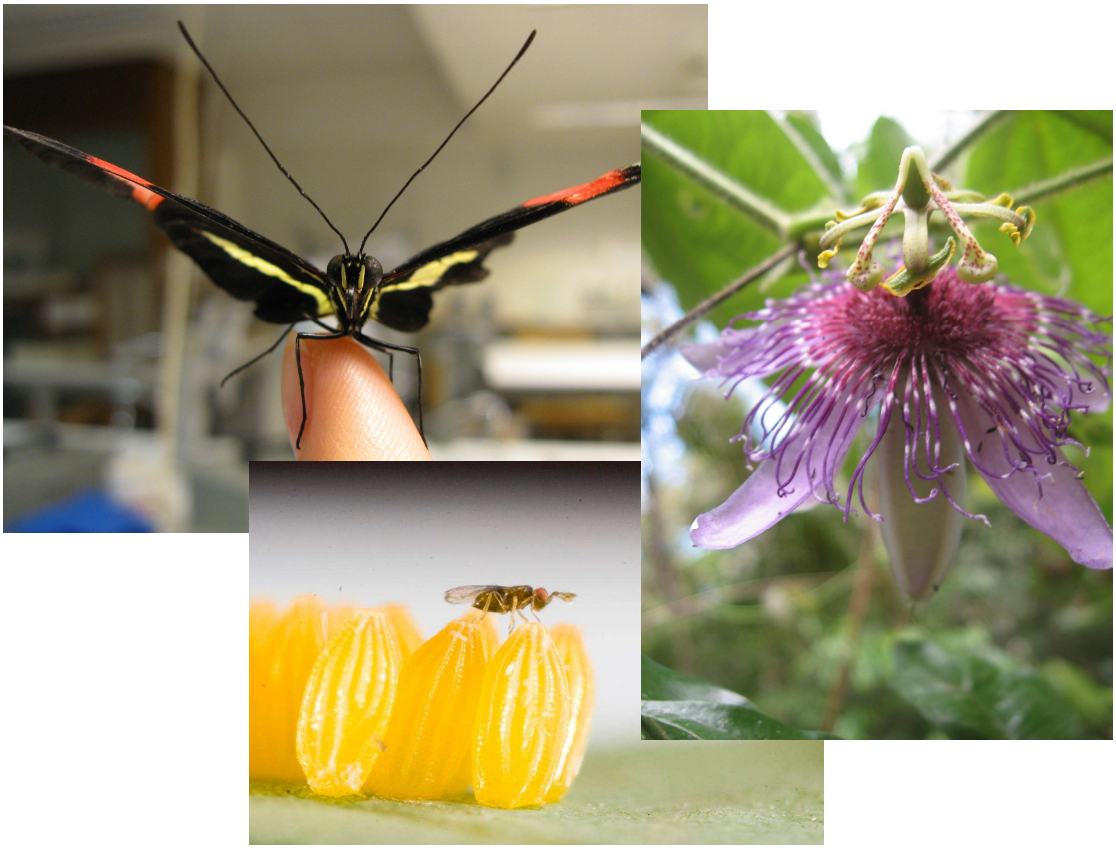
WAGENINGEN UNIVERSITY
LABORATORY OF ENTOMOLOGY

Hitch-hiking behaviour of egg parasitoids on Heliconiini butterflies in a tropical lowland rainforest



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Abstract

Egg parasitoids have evolved many different ways to find their hosts. These include the use of infochemicals from the plant or from other host stages. Chemical espionage on host sex pheromones, aggregation pheromones or anti-sex pheromones/anti-aphrodisiac pheromones is known for several egg parasitoid species. Anti-aphrodisiac pheromones are transferred from male to female during mating to render the female less attractive to other males. For an egg parasitoid an anti-aphrodisiac would be a reliable cue to spy on, because a mated female host will lay eggs. Recently, Fatouros *et al.* (2005b) showed that the egg parasitoid *Trichogramma brassicae* spies on the anti-aphrodisiac of the butterfly *Pieris brassicae* and hitch-hikes with the mated female butterfly to find her eggs. This espionage may act as a selection pressure against the use of an anti-aphrodisiac. In this study, I tried to link parasitism and hitch-hiking by egg parasitoids in the field to behavioural assays in the laboratory using an egg parasitoid community on Heliconiini butterflies in a tropical lowland rainforest in Panama as a model system. The total egg parasitism rate of Heliconiini butterflies on six *Passiflora* plant species was 16.1%. In an earlier study between 1998 and 2000 this was 12.3% (Naisbit, 2001). This indicates rather stable parasitism rates over the years. The proportion of parasitism by egg parasitoids of the genus *Ooencyrtus* (Hymenoptera; Encyrtidae) is 8.5%, by egg parasitoids of the genus *Trichogrammatoidea* (Hymenoptera; Trichogrammatidae) 3.8% and by egg parasitoids of the genus *Trichogramma* (Hymenoptera; Trichogrammatidae) 3.5%. In total a hitch-hiking proportion of 12.8% was found on 133 Heliconiini butterflies. Six hitch-hiking wasps also belonged to the same type of wasps that emerged from the collected eggs. Behavioural bioassays showed that naïve female wasps of one *Trichogramma* species probably do not spy on the anti-aphrodisiac of mated female *Heliconius melpomene* butterflies and do not specifically hitch-hike with those butterflies in nature. This study cannot conclude whether hitch-hiking egg parasitoids of Heliconiini butterflies constrain the use of an anti-aphrodisiac by the butterflies but represent a first step towards understanding whether this is the case. Further research consisting of more extensive field work and laboratory bioassays with other combinations of butterflies and parasitoid wasps needs to be carried out.

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Introduction

Parasitoids of herbivorous insect larvae are known to make use of plant volatiles that are emitted when larvae feed on the plant (Turlings *et al.*, 1990). By using plants with little volatile production, or by leaving the location where the volatiles are being emitted, herbivorous larvae may escape from parasitism (Vet and Dicke, 1992). Egg parasitoids face an enormous challenge to find their hosts. Eggs do not feed on a plant, excrete faeces or produce intense long-range odours (Fatouros *et al.*, 2008a). Egg parasitoids are known to use different cues to find the host. They are known to sometimes use plant cues. Plants can also emit volatiles in reaction to egg deposition (overview in Hilker and Meiners, 2002). This is, for example, the case in the field elm *Ulmus minor*. This is the host plant of the elm leaf beetle (*Xanthogaleruca luteola*) and before this beetle oviposits, it first gnaws shallow grooves into the leaf. The eggs are laid into these grooves and attached to the leaf with an oviduct secretion. The oviduct secretion together with the damage of the leaf, cause the plant to produce volatiles. These volatiles are attractive to the egg parasitoid *Oomyzus gallerucae* (Meiners and Hilker, 2000).
The same happens when the pine sawfly *Diprion pini* lays its eggs in a needle of *Pinus sylvestris* after slitting it. This damage to the plant together with an oviduct secretion cause the plant to produce volatiles that attract *Chrysonotomyia ruforum*, an egg parasitoid (Hilkers *et al.*, 2002). These are examples of long range infochemicals (Fatouros *et al.*, 2008) but parasitoids are also able to use close range infochemicals from plants ((Fatouros *et al.*, 2005a and Fatouros *et al.*, 2008a). These are signals that are used by parasitoids when they arrive on the plant (Fatouros *et al.*, 2008a). Examples are the wax layer of the leaf that changes chemically in reaction to the laid eggs (Little *et al.*, 2007) or a reduction in the photosynthetic activity by the leaf caused by egg deposition (Schröder *et al.*, 2005).

Egg parasitoids can also make use of other host stages (for example the adult) to find the right host stage (the eggs) (Vet and Dicke, 1992). In close range, they can detect traces that were left behind by the adult host. For example wing scales of butterflies or chemical traces of walking bugs (review in Fatouros *et al.*, 2008a). In long range, they can perform espionage on intraspecific host communication

(Fatouros *et al.*, 2008) like, for example, chemical espionage on sex pheromones, aggregation pheromones or anti-sex pheromones (Fatouros *et al.*, 2008a). Sex pheromones are being used by one sex to stimulate behavioural reaction(s) by the other sex on behalf of reproduction (Shorey, 1973). An aggregation pheromone causes other members of the same species to aggregate in a particular area (Shorey, 1973). One example is espionage on an aggregation pheromone of the bean bug *Riportus clavatus* by the egg parasitoid *Ooencyrtus nezarae*. The male bugs produce a pheromone that attracts male and female adults, as well as a specific nymph stage what attracts the nymphs to the host plant so they can eat. However, females of *Ooencyrtus nezarae* also use this pheromone to locate the host community and find host eggs (Leal *et al.*, 1995). Anti-sex pheromones are also called anti-aphrodisiac pheromones (Happ, 1969). Gilbert (1976) suggested that male *Heliconius erato* butterflies transfer an anti-aphrodisiac to the female during mating. This anti-aphrodisiac makes the female less attractive to other males that want to mate with her. This is an advantage for the male that mated with the female, because of reduced sperm competition (Gilbert, 1976 and Schulz *et al.*, 2008). For the other males, using an anti aphrodisiac may also be an advantage because they do not have to spend time and energy in courtship with a mated female (Gilbert, 1976). The mated female will not be harassed by males, which saves time and energy too (Andersson *et al.*, 2000; Bateman *et al.*, 2006). These anti-aphrodisiac pheromones would be a reliable cue to spy on for egg parasitoids, because the female host with the anti-aphrodisiac is mated, so she will lay eggs.

This transfer of anti-aphrodisiac pheromones is discovered in several butterfly species like *Heliconius melpomene* (Schulz *et al.*, 2008), *Pieris napi* (Andersson *et al.*, 2000), *Pieris brassicae* and *Pieris rapea* (Andersson *et al.*, 2003). Fatouros and co-workers (2005b) showed that the egg parasitoid *Trichogramma brassicae* (Hymenoptera; Trichogrammatidae) spies on the anti-aphrodisiac benzyl cyanide of the large cabbage white *Pieris brassicae*. *T. brassicae* wasps are approximately 0.5 mm long and are assumed to have a limited flight capability (Romeis *et al.,* 2005). Using two-choice olfactory bioassays and synthetic anti-aphrodisiac compounds, they proved that female *T. brassicae* wasps use the anti-aphrodisiac of *P. brassicae* as a foraging cue. When the parasitoids detect an anti-aphrodisiac, they still need to reach the place where the mated female butterfly is going to lay her eggs. Because of their

limited flight capability, this can be a problem. To overcome this, *T. brassicae* use the mated butterfly as a transport vehicle towards freshly laid eggs (Fatouros *et al.,* 2005b). Using two-choice mounting bioassays and a synthetic anti-aphrodisiac, Fatouros and co-workers (2005b) proved that *T. brassicae* wasps specifically mount mated female butterflies and virgin female butterflies painted with 2 µg synthetic anti-aphrodisiac. After releasing mated female butterflies carrying a wasp in a flight chamber, 50% of the wasps were found on the butterfly after it had landed on a cabbage plant, 14.2% also climbed down the butterfly and reached the host plant and 7.1% even parasitized the freshly laid eggs (Fatouros *et al.*, 2005b).

The strategy of chemical espionage in combination with hitch-hiking on a mated female host may be a more common strategy in egg parasitoids. A butterfly can reduce its own fitness by using these specific pheromones that parasitoids spy on. (Hilker and Meiners, 2002a). In this way, the parasitoids can constrain the evolution of the intraspecific communication of the butterfly (Fatouros *et al.*, 2005b). There may be a selection for minimizing the use of a pheromone or even the use of another compound on which the parasitoids cannot spy. To understand whether hitch-hiking parasitoids seriously constrain the evolution of the intraspecific communication of the host, one needs to study hosts that are known to use anti-aphrodisiacs and that are attacked by egg parasitoids of which natural parasitism rates can easily be determined. Unfortunately, eggs of pholyphagous cabbage white butterflies are difficult to find in natural systems.

Natural associations between Heliconiini butterflies and their egg parasitoids are an excellent study system. Besides the presence of an anti-aphrodisiac in *Heliconius erato* (Gilbert, 1976), Schulz *et al.* (2008) recently found that *H. melpomene* males transfer a mixture of components to the female during mating. This mixture contains (E)-β-Ocimene, which makes the female unattractive to other males (Schulz *et al.*, 2008). This butterfly species was also studied in a field research on Heliconiini butterflies and a few species from other butterfly tribes in a tropical lowland rainforest in Panama (Naisbit, 2001). Figure 1 shows that *H. melpomene* eggs are found on three *Passiflora* species. These eggs are found on *Passiflora auriculata, P. vitifolia* and mainly on *P. menispermifolia*. Five more butterfly species use *P. menispermifolia* as larval host plant (Figure 1). Interestingly, 8.72% of the eggs found on this host

plant (*P. menispermifolia*) were parasitized by egg parasitoids (Naisbit, 2001), thereby suggesting that *H. melpomene* eggs are attacked by egg parasitoids.

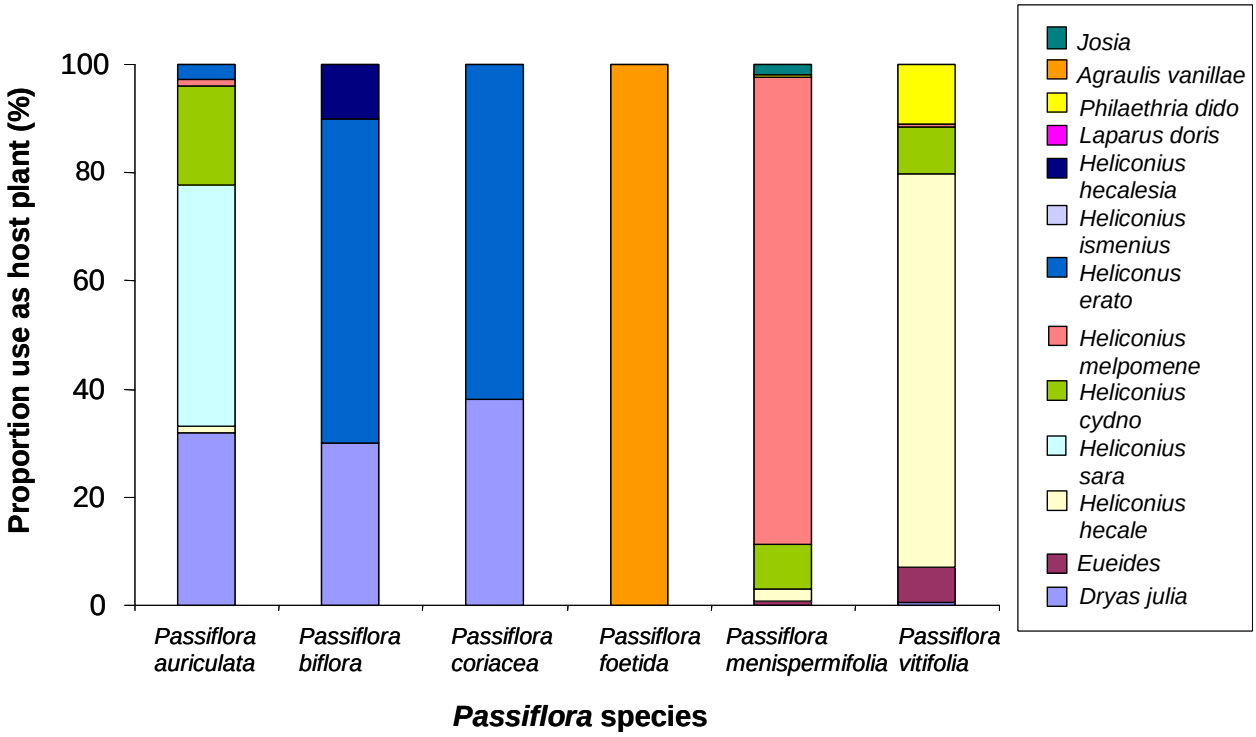

Figure 1. Host plant records from *Passiflora* species. Figure made with data from Naisbit (2001).

In total three families of egg parasitoids (namely Trichogrammatidae, Encyrtidae and Scelionidae) were found to parasitize Heliconiini eggs in this Panamanian rainforest, but their relative importance as mortality factor and their genus and species identity were not determined (Naisbit, 2001).

In this study, I tried to answer the following more specific research questions:

A. To what extent do egg parasitoids attack Heliconiini eggs in the field?

1. collection of butterfly eggs
2. rearing and identification of parasitic wasps

B. Do wasps hitch-hike with adult butterflies to find their eggs?

3. collection of adult butterflies with special butterfly nets
4. one-choice mounting bioassays

C. Does a *Trichogramma* species recognize mated female *Heliconius melpomene* butterflies by an anti-aphrodisiac?

5. two-choice olfactory bioassays

Material and methods

A. To what extent do egg parasitoids attack Heliconiini eggs in the field?

1. collection of butterfly eggs

Heliconiini butterflies have a few specific host plants on which they lay their eggs. These plants differ between the different butterfly species, but there is some overlap (Naisbit, 2001). In the field we (in the field I worked together with Joop Woelke) first searched for these *Passiflora* plants. In total we found 122 plants: 17 *P. auriculata* plants, 16 *P. biflora* plants, 17 *P. coriacea* plants, 5 *P. foetida* plants, 13 *P. menispermifolia* plants and 54 *P. vitifolia* plants. These plants were found at five different research locations in the Republic of Panama, Province Panamá: Pipeline Road (Camino del Oleoducto), Oil bunkers (near Pipeline Road, parallel to the Panama Canal), Gamboa, Sendero la laguna and Camino de la Plantación. These locations are all located in or near Soberanía National Park. The plants were found besides the trails or up to approximately 2 meters into the forest. We marked the plants with red labels and checked every plant once a week. When there was an egg on a plant, we collected it and noted the date of collection and the plant on which it was found. In the laboratory in the school building of the Smithsonian Tropical Research Institute in Gamboa the eggs were checked every day for caterpillar or wasp emergence. When a caterpillar emerged, it was placed back on a plant of the same species on which the egg was found. When a wasp(s) emerged and it was a Trichogrammatidae, Scelionidae or Encyrtidae, we tried to rear them. Sometimes this did not work out, so we put every wasp in 95% alcohol for species identification. When a wasp(s) emerged and it was not belonging to the families Trichogrammatidae, Scelionidae or Encyrtidae, it was put in alcohol immediately. The fieldwork was done in a period of ten weeks (February 3rd until April 12th 2008).

2. rearing and identification of parasitoid wasps

In Gamboa the conditions in the laboratory were not optimal for rearing the collected wasps, mainly due to the low humidity. For parasitoid rearing about ten *Heliconius erato* and *H. melpomene* eggs were used that were obtained from rearings at STRI (maintained by Richard Merril and Moises Abanto Flores). A few *Trichogramma* lines were set up and maintained successfully. They were sent to the Laboratory of Entomology in Wageningen. Rearing of these lines in Wageningen worked out well. Here, the rearing took place in a climate chamber at 22˚C and 62% RH with a light period of 16 hours and a dark period of 8 hours. Besides this, some collected Heliconiini eggs were shipped directly to Wageningen. Unfortunately, it was impossible to rear Encyrtid and Scelionid wasps, not on *Heliconius erato* and *H. melpomene* eggs in Panama, nor on *Ephestia kuehniella* or *Mamestra brassicae* eggs in Wageningen. The Trichogrammatid wasps were doing well on both *Ephestia* eggs and *Mamestra* eggs. After two generations I only used *Ephestia* eggs because these are easier to use.

The identification of the wasps other than Trichogrammatidae was done morphologically by Yde Jongema (Laboratory of Entomology, Wageningen) and Dr. John Noyes (British Museum of Natural History, London) specifically looked at the Encyrtid wasps. The identification of Trichogrammatid wasps was done molecularly using a method based on ITS2 DNA sequences (Stouthamer *et al.* 1999):

<u>DNA extraction</u>

DNA extraction was done using the CHELEX method. After an individual wasp was taken out of the alcohol and dried on filter paper, it was grinded with a closed pasteur pipette. Fifty µl of 5% CHELEX solution and 4 µl of proteinase K was added and the samples were incubated overnight at 56˚C. They were stored at -20˚C.

<u>PCR amplification</u>

The PCR mix included the following per sample: 14.875 µl H_2O, 5.0 µl GoTaq buffer, 1.5 µl $MgCl_2$, 0.5 µl dNTP (10 mM), 0.5 µl forward primer, 0.5 µl reverse primer, 0.125 µl GoTaq polymerase (5 units/µl) and 2.0 µl DNA extract. The PCR amplification was done in a 0.2 ml thin-wall Eppendorf tube.

The used ITS2 primers were:
5'-TGTGAACTGCAGGACACATG-3' (forward)
5'-GTCTTGCCTGCTCTGAG-3' (reverse)
The PCR program was as follows: 3 min. at 94 ˚C followed by 33 cycles of 40 sec. at 94 ˚C, 45 sec. at 53 ˚C and 45 sec. at 72 ˚C. After the last cycle the samples were kept for 5 min. at 72 ˚C. The PCR products were stored at 4 ˚C for immediate Gel Electrophoresis or at -20 ˚C in the freezer.

Agarose Gel Electrophoresis

The PCR products were run at 80 Volt for 50 minutes on a 1.0% Agarose gel stained with ethidium bromide.

Cloning and sequencing

Extraction of DNA from gel

Per PCR product 20 µl of the PCR product was used for Agarose Gel Electrophoresis. The DNA fragments were cut out of the gel and the DNA was cleaned from gel using the MinElute® Gel Extraction Kit (QIAGEN; protocol according to manufacturer).

Ligation

Three µl of extracted DNA was mixed with 5 µl Rapid Ligation Buffer, 1 µl T4 DNA Ligase and 1 µl pGEM® -T Easy Vector. This was incubated overnight at -4˚C.

Transformation

Two µl of the ligation product was added to 20 µl of XL2-Blue competent cells (Stratagene). These samples were left on ice for 30 minutes. After 30 minutes, the cells need to be heat shocked. This was done by placing the tubes with the cells for exactly 30 seconds in a water bath of 42 ˚C and then placing the tubes on ice for 2 minutes. After that, 970 µl of SOC medium of 42 ˚C was added to the samples and this was incubated for 1 hour at 37˚C, shaking at 225 rpm.
Hundred µl of the samples was plated on agar plates under sterile conditions. The rest of the sample was centrifuged to get a pellet of cells at the bottom. Then 750 µl of the supernatant was removed. The cells were dissolved in the left over of the supernatant by pipetting up and down. Hundred µl of this sample was plated on an

agar plate. All plates were incubated upside down overnight at 37 ˚C. The agar plates were made with the following ingredients for 1 liter: 10 g Trypton, 5 g Yeast, 5 g NaCl, 15 g of agar, 1 liter of water. A bottle with these ingredients was autoclaved and cooled down to around 60 ˚C. One ml ampicillin (100mg/ml), 2 ml X-gal (20 mg/ml) and 1 ml of IPTG (100mM) was added and the mixture was poured out on Petri dishes. The next day white colonies on the plates were picked with a sterile wooden toothpick and put into an autoclaved tube with 3.5 ml LB medium and with 3.5 µl ampicillin (100mg/ml). These tubes were incubated overnight at 37˚C, shaking at 250 rpm.

Miniprep

To extract the DNA from the cells a Gen Elute™ Plasmid Miniprep Kit was used (Sigma-Aldrich; protocol according to manufacturer).

Control for correct insertion

To check whether the transformation went well, a PCR reaction was done. The mix was made the same as before, except for the DNA sample. Because of the high concentration of the DNA sample after the Miniprep, only 0.5 µl was used instead of 2.0 µl. To compensate 1.5 µl H2O was used extra.

Sequencing

The concentration of the Miniprep products was measured in a spectrophotometer by using 100 µl of a dilution of the Miniprep product (2.0 µl Miniprep product/ 98 µl H_2O). The concentration had to be 100 µg/ml or higher. Eurofins MWG GmbH requested 1200 µg of Miniprep product, so the right amount of product was put in tubes, dried and send to Eurofins MWG GmbH. The sequences were aligned and clipped using the programme Vector NTI Advance 10.

Blasting

The aligned and clipped sequences were blasted and compared with known ITS2 sequences in GenBank. When similarities were low, sequences were send to Prof. Dr. Richard Stouthamer (Department of Entomology, University of California Riverside), who compared them with his more extensive *Trichogramma* ITS2 database.

B. Do wasps hitch-hike with adult butterflies to find their eggs?

3. collection of adult butterflies with special butterfly nets

While checking the *Passiflora* plants in the fields, we also collected adult butterflies of the tribe Heliconiini with a butterfly net. We caught several individuals of the following species*: Heliconius melpomene, Heliconius hecale, Heliconius erato, Heliconius ismenius, Heliconius cydno, Heliconius sara, Heliconius sapho, Heliconius doris, Philaethria dido, Agraulis vanillae, Dione juno, Dryas iulia, Eueides lybia Olympia* and *Eueides aliphera gracilis*. The butterfly nets had a vial in the bottom. This vial should prevent many wasps to get lost in the net. After catching the butterfly in the vial, the vial was closed with a lid and replaced by a new one. In the laboratory the vials with the butterflies were put overnight in a refrigerator. The next morning the vials were taken out of the refrigerator and the butterflies and vials were examined for the presence of egg parasitoid wasps using a dissection microscope. When a wasp was found on the butterfly or in the vial, it was put in alcohol (when it did not belong to the Trichogrammatidae) or we tried to set up a line with the wasp (when it belonged to the Trichogrammatidae). The butterflies were sexed and used for species identification. Afterwards the butterflies were fed with honey water and released around the place where they were caught.

4. one-choice mounting bioassays

To test whether *Trichogramma* wasps emerging from Heliconiini eggs climb on butterflies, a one-choice mounting bioassay was carried out at the Laboratory of Entomology in Wageningen with the following setup: A virgin butterfly was placed in a mounting arena on one side and a female wasp on the other side (with approximately 2 cm in between) (complete description in Fatouros *et al.* 2005b). For 300 seconds the wasp was observed. When the wasp climbed on the butterfly the time until the wasp climbed onto the butterfly and the time that it remained on the butterfly was measured. The body part of the butterfly on which the wasp climbed was noted, as well as the flying activity of the wasps. Per combination 40 to 45 wasps were tested; with a maximum of 15 individuals per wasp species per butterfly species per day. With each individual butterfly only 3 wasps were tested. The position of the next

butterfly was different from the butterfly before to prevent preferences of the wasps for one side of the arena.

One-choice bioassays were done with *Trichogramma* wasps from Panama in combination with *Heliconius melpomene, Pieris brassicae* and *Pieris rapae* butterflies. I had to choose one *Trichogramma* line that was found on *P. foetida* because this was the line with the highest amount of wasps. At the time of the behavioural experiments, the species identity of this *Trichogramma* sp1 line was unknown. *Trichogramma* sp1 wasps were tested in combination with *H. melpomene* to see if the wasps use this butterfly species for hitch-hiking. One-choice bioassays were also done with *T*. sp1 in combination with *P. brassicae* and *P. rapae* to see whether *T*. sp1 wasps are specialized to one butterfly species or generally climb onto butterflies, even on two cabbage white butterfly species that they will never encounter in the field. One-choice bioassays were also done with *Trichogramma brassicae* and *Trichogramma evanescens* wasps in combination with *H. melpomene* butterflies to compare their hitch-hiking behaviour with that of *T.* sp1. The palearctic species *T. brassicae* and *T. evanescens* will never encounter *H. melpomene* in the field. Unfortunately, it was not possible to obtain *H. melpomene* matings in our small cages in Wageningen, so in all bioassays I had to use only virgin female butterflies.

C. Does a *Trichogramma* species recognize mated female *Heliconius melpomene* butterflies by an anti-aphrodisiac?

5. Two-choice olfactory bioassay

To test whether *T.* sp1 wasps show a preference for the anti-aphrodisiac of *H. melpomene* butterflies, an olfactory bioassays was carried out using the following set-up: an arena with two compartments, in each compartment I placed one butterfly as an odour source (complete description in Fatouros *et al.* 2005b). The following combination was tested: a virgin female *H. melpomene* painted with 10 µl of the synthetic anti-aphrodisiac β-Ocimene dissolved in hexane (0.2 µg β-Ocimene / µl hexane) against a virgin *H. melpomene* female painted with 10 µl hexane only. On top of the compartments a gauze was present on which a female *T*. sp1 wasp was released. For 300 seconds it was recorded how long the wasp spent above each odour source.

Keeping the *Heliconius melpomene* butterflies

Heliconius melpomene rosina pupae that originate from Central America were ordered from The Stratford Butterfly Farm in the UK. It is important to keep the pupae warm and humid. They were kept in small cages at 28 ˚C and 68% RH. When the pupae hatch, they need something to hold and later to hang upside down, in order to dry their wings in the right position. In captivity in small cages, the butterflies do not always feed themselves and they do not mate. To keep the butterflies alive they need to be hand fed with honey water every day. In the cage I also placed a couple of cups with honey water and sometimes I saw them drinking by themselves. Honey water includes amino acids (C. Estrada personal communication). In nature the butterflies eat pollen which also include amino acids (Gilbert, 1972).

Statistical analysis

Comparisons between egg parasitism rates were done using multiple Chi-square tests with a 2x2 contingency table ($\alpha<0.05$). Comparisons between the proportions of climbing *Trichogramma* wasps in the one-choice mounting bioassays were done with multiple Chi-square tests with a 2x2 contingency table ($\alpha<0.05$). Comparisons between the proportions of flying wasps in the one-choice mounting bioassays were done with multiple Chi-square test with a 2x2 contingency table ($\alpha<0.05$). Residence times above the two odour fields in the olfactory bioassay were compared with a Wilcoxon's matched pairs signed rank test ($\alpha<0.05$).

Results

A. To what extent do egg parasitoids attack Heliconiini eggs in the field?

In figure 2 the egg parasitism rates (%) per *Passiflora* species found by Naisbit (2001) are shown. Parasitism ranged from 6.3% on *P. coriacea* to 15.8% on *P. vitifolia*. The total egg parasitism rate was 12.3%. Eggs were only collected at Pipeline Road. No eggs were found on *Passiflora foetida* (Naisbit 2001).

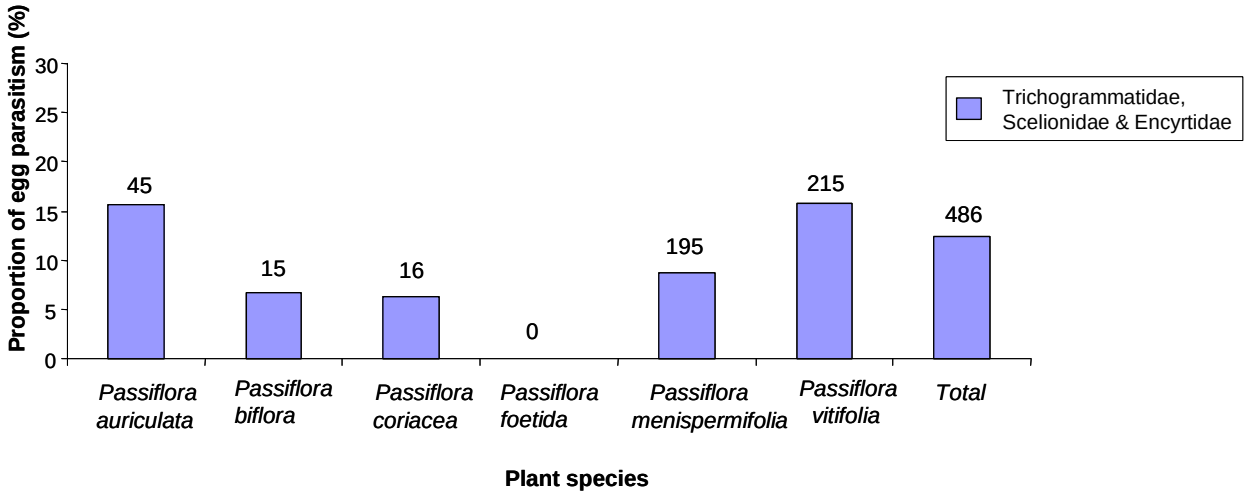

Figure 2. Egg parasitism rates per *Passiflora* plant species. Figure made with data from Naisbit (2001). The data are obtained between August 1998 and March 2000. The bars indicate the proportion of eggs that was parasitized by Trichogrammatidae, Scelionidae and Encyrtidae wasps. Above the bars the number of collected eggs is shown. Number of investigated plants per *Passiflora* species was unknown (Naisbit 2001).

In 2008, we mainly collected at Pipeline Road and at a few other locations near to Pipeline Road. The total egg parasitism rate in 2008 is 16.1% (Figure 3). Highest parasitism was found on *P. bifora* (26.1%).

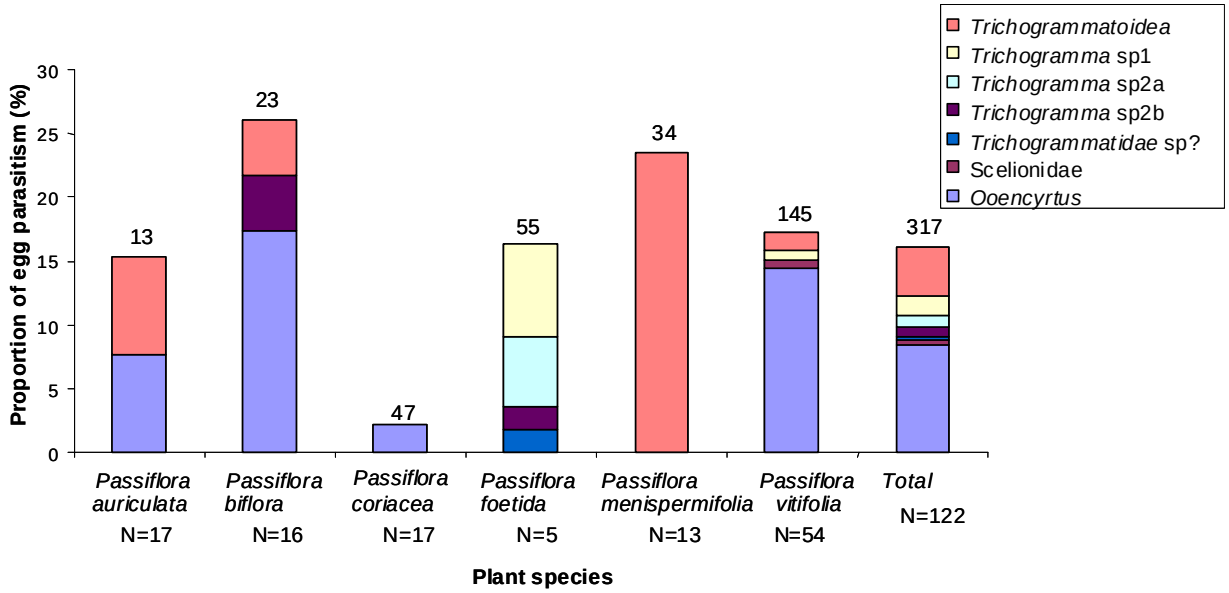

Figure 3. Percentage of egg parasitism per *Passiflora* plant species in 2008. The bars indicate the proportion of eggs that was parasitized by *Trichogrammatoidea*, *T.* sp1, *T.* sp2a, *T.* sp2b, Trichogrammatidae sp?, Scelionidae or *Ooencyrtus*. On the x-axis the plant species are indicated with the number of plants of each species (N). Above the bars the number of collected eggs is shown.

There were no significant differences in the parasitism rates per plant species between the two different field studies for 4 out of 5 *Passiflora* species on which egg parasitism was observed. The total egg parasitism rates are also not significantly different between the years (Table 1)

Table 1. P values resulting from comparisons between parasitism rates per *Passiflora* species in the two different field studies using a chi-square test with a 2x2 contingency table with α<0.05. In the study of Naisbit (2001), no eggs were found on *P. foetida*.

***Passiflora* species**	*Passiflora auriculata*	*Passiflora biflora*	*Passiflora coriacea*	*Passiflora foetida*	*Passiflora menispermifolia*	*Passiflora vitifolia*	Total
P value	0.988	0.131	0.417	-	0.011	0.720	0.133

In 2008, parasitism was found by *Trichogrammatoidea* (3.79%) (Hymenoptera; Trichogrammatidae), *Trichogramma* sp1 (1.58%) (Hymenoptera; Trichogrammatidae), *Trichogramma* sp2a (0.95%) (Hymenoptera; Trichogrammatidae), *Trichogramma* sp2b (0.63%) (Hymenoptera;

Trichogrammatidae), Trichogrammatidae *sp*? (0.32%) (Hymenoptera; Trichogrammatidae), Scelionidae (0.32%) (Hymenoptera; Scelionidae) and *Ooencyrtus* (8.52%) (Hymenoptera; Encyrtidae). Of all Trichogrammatid wasps, ITS2 genes were amplified and visualized on an agarose gel (Figure 4). After sequencing these genes, none of the ITS2 sequences were identical to already known ITS2 sequences of *Trichogramma* wasps (appendix 1). At least one species of *Trichogrammatoidea* was found emerging from Heliconiini eggs. Besides this, at least 2, and possibly even 4, species of *Trichogramma* were found. *Trichogramma* sp2a and *Trichogramma* sp2b may, or may not, be the same species. The ITS2 sequence of Trichogrammatidae sp? failed but this wasp definitely belonged to the family Trichogrammatidae (and most likely the genus *Trichogramma*) based on morphology. *Trichogramma* sp1 is the species used in subsequent behavioural experiments. This species was mainly found on *P. foetida* and once on *P. vitifolia. Trichogramma* sp2a is only found on *P. foetida*. *Trichogramma* sp2b is only found on *P. foetida* and *P. biflora*. The species identity of the Scelionid and Encyrtid wasps is still unknown at the time of writing this thesis.

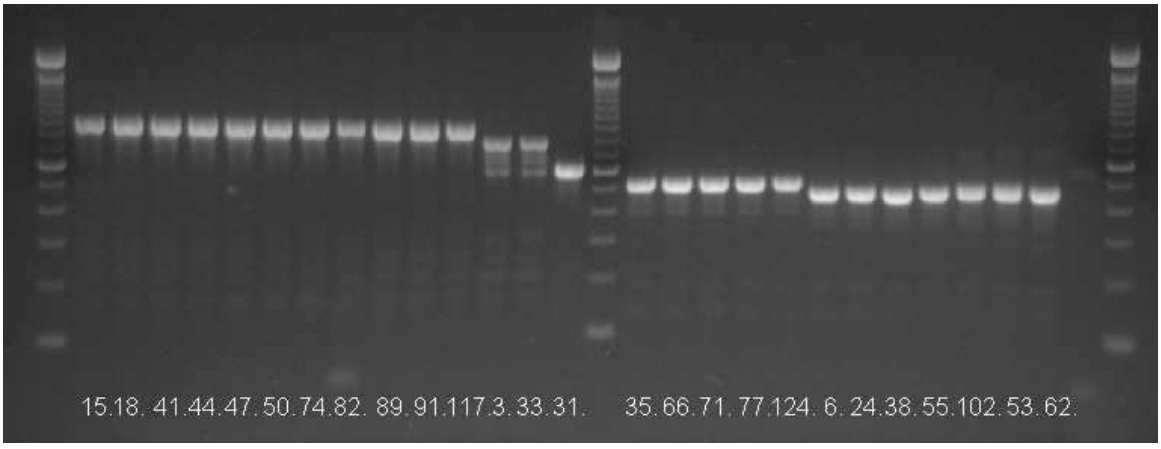

Figure 4. ITS2 products of wasps belonging to the family Trichogrammatidae, run on a 2% agarose gel that was stained with ethidium bromide. The marker used is a 100bp ladder.

- **The genus *Trichogrammatoidea* (15, 18, 41, 44, 47, 50, 74, 82, 89, 91, 117)**
- **Two fragments that belong to a genus related to *Trichogramma* (unknown genus) (3, 33 (found on butterflies))**
- ***Trichogramma iracildae* (31 (found on a butterfly))**
- ***Trichogramma* sp1 (35, 66, 71, 77, 124)**
- ***Trichogramma* sp2a (6 (found on a butterfly)), 38, 55, 102)**
- ***Trichogramma* sp2b (53, 62)**
- **Trichogrammatidae sp? (24)**

B. Do wasps hitch-hike with adult butterflies to find their eggs?

In the field 133 butterflies of the tribe Heliconiini were caught. On 12.8 % of these butterflies one parasitic wasp was found (17 out of 133). Two of these 17 wasps were *Trichogramma* wasps. After comparing the ITS2 sequences with other ITS2 sequences found in this study, and ITS2 sequences from existing databases, it became apparent that one *T.* sp2a wasp was found on a female *H. hecale* butterfly and one *T. iracildae* wasp was found on a male *H. doris* butterfly. Two *Ooencyrtus* wasps were found on two female *Dryas iulia* butterflies. The other wasps belonged to the families Eulophidae, Scelionidae, Mymaridae, Braconidae, Aphelinidae and an unknown genus related to *Trichogramma* (Figure 5).

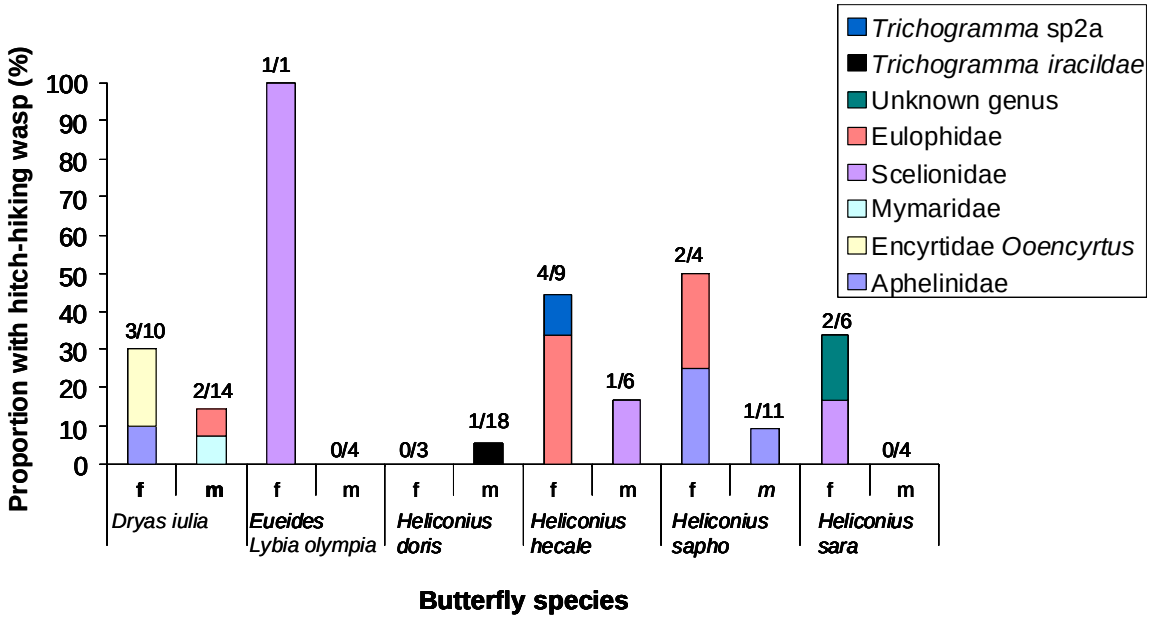

Figure 5. Proportion of butterflies with a hitch-hiking wasp per butterfly species. Only the butterfly species are shown on which a parasitic wasp was found. On the x-axis, "f" stands for female and "m" stands for male. Above each bar the number of hitch-hiking wasps and the number of caught butterflies is indicated.

Most wasps are found on female butterflies, but 4 wasps were also found on male butterflies.

In the one-choice mounting bioassays, *Trichogramma* sp1 and *Trichogramma brassicae* climbed significantly more often onto the two *Pieris* species than onto the *Helcionius melpomene* butterflies (Figure 6). *T. evanescens* climbed significantly more often on *P. brassicae* than on *Heliconius melpomene* but did not distinguish between climbing onto *P. rapae* and *H. melpomene*. No significant differences were found in the proportion of wasps climbing onto *H. melpomene* between the three wasps species.

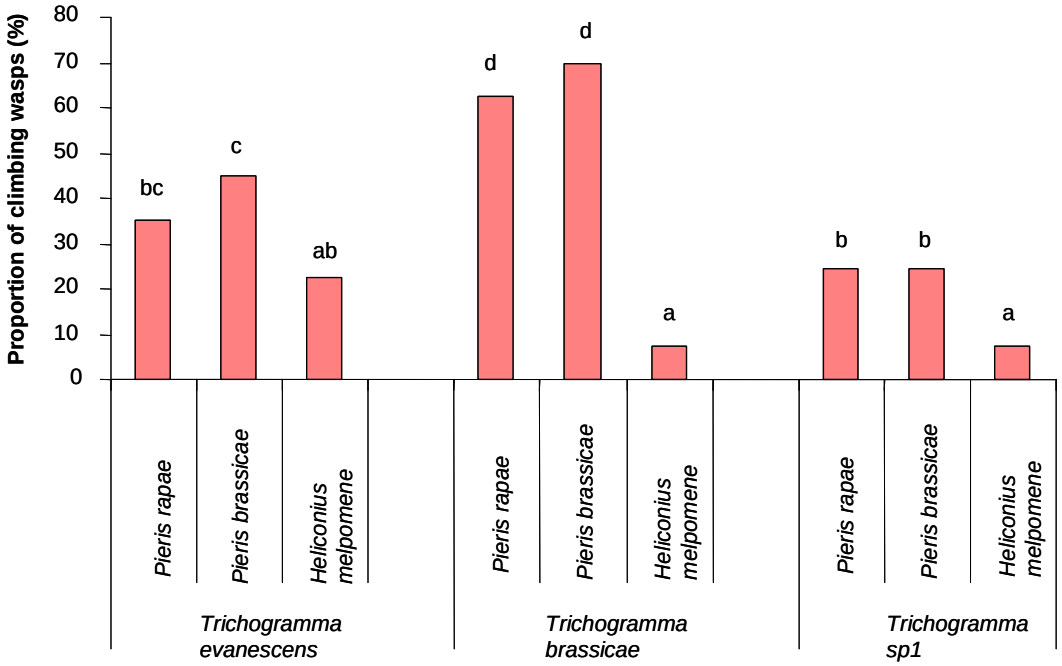

Figure 6. One-choice mounting assays. On the x-axis three different *Trichogramma* species are combined with three different butterfly species. The data from the test combinations of *T. evanescens* against *P. rapae* and *P. brassicae*, and *T. brassicae* against *P. rapae* and *P. brassicae* are obtained from respectively Pashalidou (2008) and Woelke (2008). The bars indicate the proportion of climbing wasps. Above the bars the significant differences are indicated. When there is no difference, the same character is used (Chi-square tests with 2x2 contingency tables, $\alpha<0.05$).

Interestingly, it was observed that *Trichogramma* sp1 fly significantly more often in the one-choice bioassay than the other two *Trichogramma* species (P<0.001).

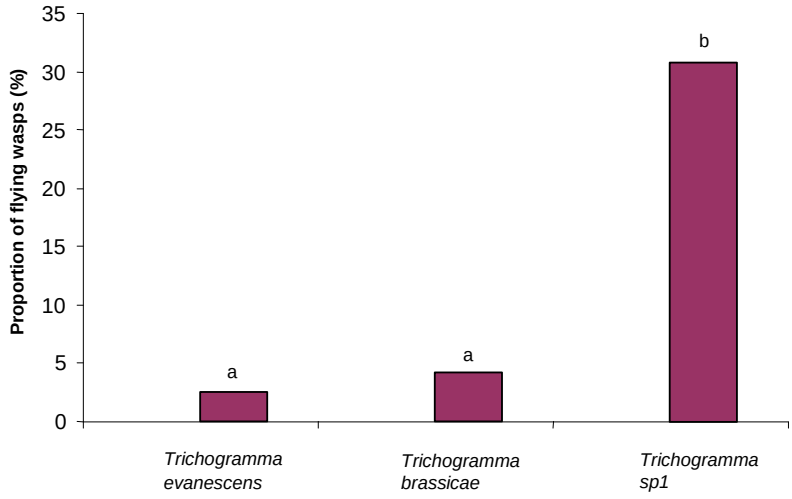

Figure 7. Proportion of wasps flying. The bars represent the percentage of wasps flying in the one-choice bioassay. Above the bars the significant differences are indicated. When there is no difference the same character is used (Chi-square test with a 2x2 contingency table, α<0.05).

C. Does a *Trichogramma* species recognize mated female *Heliconius melpomene* butterflies by an anti-aphrodisiac?

In the two-choice olfactory bioassay the wasps did not show a significant preference for the odour of a virgin female *H. melpomene* butterfly painted with β-Ocimene dissolved in hexane compared to the odour of a virgin female *H. melpomene* painted with only hexane (P=0.430; Wilcoxon's matched pairs signed rank test (α<0.05)).

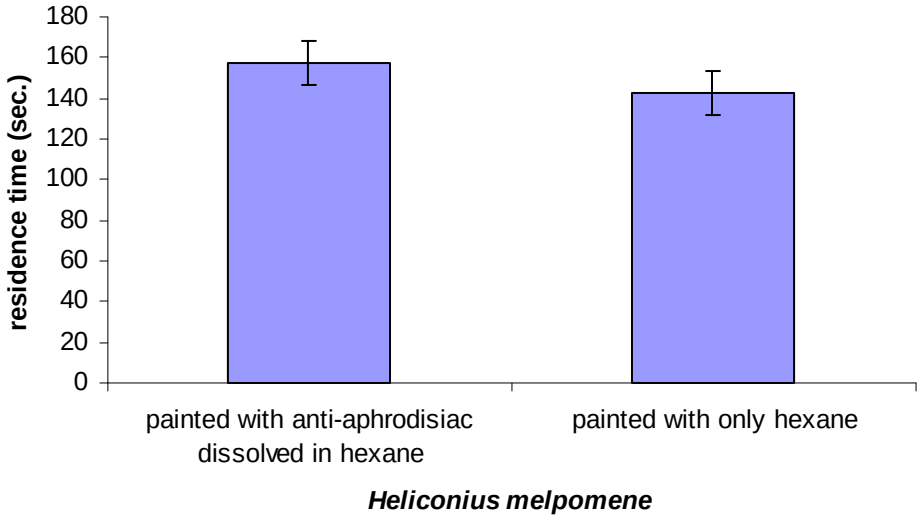

Figure 8. Results of the two-choice olfactory bioassay. On the x-axis two groups of virgin female *Heliconius melpomene* butterflies are indicated. The bars indicate the mean residence time of the *Trichogramma* sp1 wasps above each of the two butterfly groups.

Discussion

In this research, the total egg parasitism rate found on six *Passiflora* plant species was 16.1%. The total rate of egg parasitism on the same plant species in 1998-2000 was 12.3% (Naisbit, 2001). The individual parasitism rates on four of five *Passiflora* species on which egg parasitism was observed in our study and Naisbit's study (*P. auriculata, P. biflora, P. coriacea* and *P. vitifolia*) did not differ between the two field surveys, even though the work was carried out in different seasons. This study was carried out in the dry season whereas Naisbit (2001) did his survey in both the dry and the wet season. Woelke (unpublished data) showed rather stable parasitism rates in ten weeks of the dry season in 2008. All in all, the results from both studies indicate rather stable parasitism rates over time.

Egg parasitoids of the genera *Ooencyrtus* (Encyrtidae; 8.5%) and *Trichogrammatoidea* (Trichogrammatidae; 3.8%) are responsible for the highest proportions of parasitism, followed by wasps of the genus *Trichogramma* (Trichogrammatidae; 3.5%). When comparing the parasitism rates by *Trichogramma* wasps with other studies on natural *Trichogramma*-host associations, parasitism rates of *Heliconius* eggs seem rather low. Two other studies on other natural systems namely found 42.1% (during three months of collecting eggs; Huigens, 2003) and 24.7% (during two months of collecting eggs; Simpson, 2007) egg parasitism by *Trichogramma*. It was impossible to determine the species identity of the butterfly eggs. The plant species on which the eggs were found can, however, also tell us something about the possible host range of the different egg parasitoids because Naisbit (2001) has described the host plants for almost all Heliconiini butterflies investigated in this study:

Trichogrammatoidea wasps were found on *Passiflora auriculata, Passiflora biflora, Passiflora menispermifolia* and *Passiflora vitifolia*. This implies that they may parasitize all butterflies from figure 1 except for *Agraulis vanillae*.

***Trichogramma* sp1** wasps were found on *P. foetida* and *P. vitifolia*. In theory they could then parasitize eggs of *Agraulis vanillae, Philaethria dido, Heliconius melpomene, Heliconius cydno, Heliconius hecale, Eueides* and *Dryas iulia*.

***Trichogramma* sp2a** wasps were found on *P. foetida* implying that they may parasitize *Agraulis vanillae.*
***Trichogramma* sp2b** wasps were found on *P. biflora* and *P. foetida* implying that they may parasitize *Augraulis vanillae, Heliconius hecalesia, Heliconius erato* and *Dryas iulia.*
Trichogrammatidae sp? wasps were found on *P. foetida* implying that they may parasitize *Agraulis vanillae*
Scelionid wasps were found on *P. vitifolia* implying that they may parasitize *Philaethria dido, Heliconius melpomene, Heliconius cydno, Heliconius hecale, Eueides* and *Dryas iulia*.
Ooencyrtus wasps were found on *P. auriculata, P. biflora, P. coriacea* and *P. vitifolia* implying that they may parasitize all butterflies from figure 1 except for *Agraulis vanilae* and *Josia.*
This has to be treated with care because the frequency of host plant use by the butterflies is not taken into account. Even though different butterfly species can lay eggs on the same plant species, some species do that much more often than others (Naisbit 2001; Figure 1).

In this research on 12.8% of 133 caught Heliconiini butterflies a parasitic wasp was found. Six of these hitch-hiking wasps also belonged to the same type of wasps that emerged from the collected eggs; 2 *Ooencyrtus* wasps were found on 10 female *Dryas iulia* butterflies (20.0%), 1 Scelionid wasp was found on 1 female *Eueides lybia Olympia* butterfly (100%), 1 Scelionid wasp was found on 6 male *H. hecale* butterflies (16.7%), 1 Scelionid wasp was found on 6 female *H. sara* butterflies (16.7%) and 1 *Trichogramma* sp2a wasp was found on 9 female *H. hecale* butterflies (11.1%). For two *Trichogramma* species on several butterfly species in the Netherlands, slightly lower proportions were found; 6.3% *Trichogramma evanescens* on 16 female *P. brassicae* butterflies, 1.1% *T. evanescens* on 90 female *P. rapae* butterflies, 0.53% *T. evanescens* on 187 male *P. rapae* butterflies, 1.08% *T. evanescens* on 93 male *P. napi* butterflies and 0.86% *Trichogramma brassicae* on 116 female *M. jurtina* butterflies (Huigens and Fatouros, unpublished data). The proportions of hitch-hiking wasps in both Panama and the Netherlands can be an underestimation of the actual proportion, because some recent evidence suggests that the method of catching butterflies that was used so far does not detect all hitch-hiking wasps (Van

Roosmalen, unpublished data). Besides this, it is possible that some butterfly individuals were wrongly identified because mimicry is very common in Heliconiini butterflies (Keith and Brown, 1981). During the fieldwork butterflies were sometimes not caught out of the sky but while sitting on a plant. This implies that some wasps that ended up in the collection vials were actually sitting on a plant and not on the butterfly. This may also explain why we collected relatively many egg parasitoid species/genera during the butterfly catches that did not emerge from the collected butterfly eggs. In this study and in previous studies (Woelke, 2008; Pashalidou, 2008) wasps were also found on male butterflies. These wasps may hitch-hike with the male host and transfer to the female host during mating.

Naïve female *Trichogramma* sp1 wasps probably do not spy on the anti-aphrodisiac of mated female *Heliconius melpomene* butterflies and do not specifically hitch-hike with mated female *Heliconius melpomene* butterflies. *Trichogramma* sp1 wasps were not found hitch-hiking on Heliconiini butterflies and in the bioassays they did not climb very frequently on the *H. melpomene* butterflies. *T*. sp1 wasps even climb significantly more often onto *P. brassicae* and *P. rapae* butterflies, even though they never encounter these butterflies in nature (Chacón and Montero, 2007). Even the two palearctic *Trichogramma* species, *T. brassicae* and *T. evanescens*, who never encounter *H. melpomene* in the field, climbed just as frequent on *H. melpomene* butterflies as *T*. sp1. Also more *T*. sp1 wasps flew in the mounting arena than *T*. sp1 wasps climbed onto a virgin female *H. melpomene* butterfly (18 of 40 wasps flying, 3 of 40 wasps climbing; P=0.001, Chi-square test with a 2x2 contingency table ($\alpha<0.05$)). This indicates that some *Trichogramma* species are better flyers than generally assumed for *Trichogramma* wasps (review in Fatouros *et al.* 2008a) and do not strongly rely on hitch-hiking.

Trichogramma sp1 wasps do not seem to spy on the anti-aphrodisiac ((E)-β-Ocimene) of *Heliconius melpomene* although only one amount of synthetic anti-aphrodisiac (2 µg) was tested in this study. This amount was chosen because it was found on the butterflies (Estrada, unpublished data) and 2 µg of the anti-aphrodisiacs of *P. brassicae* and *P. rapae* butterflies were found to be attractive for female *T. brassicae* wasps (Fatouros et al., 2005b; Woelke, 2008). *Trichogramma evanescens*

wasps are known to first need a positive learning experience to recognize their host anti-aphrodisiac (Pashalidou, 2008). This may also be the case for *T.* sp1. *Trichogramma* sp1 emerged from eggs found on *Passiflora foetida* and *Passiflora vitifolia*, while *H. melpomene* mainly use *P. menispermifolia* as a host plant. Therefore the combination of *H. melpomene* and the egg parasitoid *T.* sp1 may be one that does not occur in nature. Besides this, the *H. melpomene* butterflies used in the bioassays may be different from the *H. melpomene* butterflies in the study area in Panama.

To understand whether hitch-hiking egg parasitoids constrain the use of an anti-aphrodisiac by butterflies, natural egg parasitism and hitch-hiking rates should be combined with behavioural assays in the laboratory. For *T. evanescens* wasps it is known that they are able to associatively learn to exploit an anti-aphrodisiac of *Pieris rapae* and *P. brassicae* and hitch-hike with those butterfly species in nature (Pashalidou, 2008). However, parasitism rates of up to 30% of the *P. rapae* eggs are only found in artificially created cabbage fields (Fatouros and Huigens, unpublished data). This suggests that *T. evanescens* wasps may act as a selection pressure against the use of anti-aphrodisiac pheromones by *P. rapae* and *P. brassicae* butterflies. However, natural parasitism in both butterfly species still needs to be quantified and that is not easy because their eggs are extremely difficult to find.

This study now represents a first step to study such in a natural system in which it is rather easy to determine natural egg parasitism. This study revealed parasitism of Heliconiini eggs by at least three species belonging to the family Trichogrammatidae (7.3%), at least one species belonging to the family Scelionidae (0.32%) and at least one species belonging to the genus *Ooencyrtus* (8.5%). At least one *Ooencyrtus* species, one Scelionid species and two *Trichogramma* species were found hitch-hiking with Heliconiini butterflies. Unfortunately, it was not found whether egg parasitoids of Heliconiini butterflies also exploit anti-aphrodisiacs of their hosts in laboratory bioassays. In future experiments, *Trichogramma* sp2a and *Heliconius hecale* on which this wasp species was found hitch-hiking in nature, should be tested. Estrada, Schulz, Yildizhan and Gilbert (unpublished data) already found β-Ocimene to be present in the abdominal glands of *H. hecale*, among many other Heliconiini butterflies. No behavioural tests have been done so far to investigate

whether β-Ocimene also acts as an anti-aphrodisiac for these butterfly species. Another option would be to test *H. melpomene* in combination with another wasp species. This wasp species should be one found on *P. menispermifolia*, the main host plant of *H. melpomene*. In this research, only wasps of the genus *Trichogrammatoidea* were found on *P. menispermifolia*, although wasps of this genus were not found hitch-hiking on Heliconiini butterflies. More fieldwork is also necessary to get a better idea of which egg parasitoids hitch-hike with the butterflies in nature and to determine more exact egg parasitism rates.

Very recently, Fatouros *et al*., (2008b) reported that a butterfly anti-aphrodisiac can also trigger chemical changes in a plant and that these changes indicate the presence of butterfly eggs for *Trichogramma* wasps. It would be very interesting to study whether egg parasitoids of Heliconiini eggs could constrain the use of an anti-aphrodisiac in another way than by hitch-hiking with mated female butterflies. The high flying frequency in *T.* sp1 wasps may indicate that these wasps can find *Passiflora* plants rather easily without hitch-hiking.

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References

Andersson, J., Borg-Karlson, A-K. and Wiklund, C. (2000) Sexual cooperation and conflict in butterflies: a male-transferred anti-aphrodisiac reduces harassment of recently mated females. *Proceedings of the Royal Society of London Series B* **267**: 1271-1275

Andersson, J., Borg-Karlson, A-K. and Wiklund C. (2003) Antiaphrdisiacs in Pierid butterflies: A theme with variation! *Journal of Chemical Ecology* **29**: 1489-1499

Bateman, P.W., Ferguson, J.W.H. and Yetman, C.A. (2006) Courtship and copulation, but not ejaculates, reduce the longevity of female field crickets (*Gryllus bimaculatus*). *Journal of Zoology* **268**: 341-346

Chacón, I. and Montero, J. (2007) Butterflies and moths of Costa Rica. Instituto Nacional de Biodiversidad

Fatouros, N.E., Bukovinszkine'Kiss, G., Kalkers, L.A., Soler-Gamborena, R., Dicke, M. and Hilker, M. (2005a). Oviposition-induced plant cues: do they arrest *Trichogramma* wasps during host location? *Entomologia Experimentalis et Applicata* **104**: 181-192

Fatouros, N.E., Huigens, M.E., van Loon, J.J.A., Dicke, M. and Hilker, M. (2005b). Chemical communication: Butterfly anti-aphrodisiac lures parasitic wasps. *Nature* **433**: 704

Fatouros, N.E., Dicke, M., Mumm, R., Meiners, T. and Hilker, M. (2008a) Foraging behaviour of egg parasitoids exploiting chemical information. *Behavioral Ecology* **19**: 677-689

Fatouros, N.E., Broekgaarden, C., Bukovinszkine'Kiss, G., van Loon, J.J.A., Mumm, R., Huigens, M.E., Dicke, M. and Hilker, M. (2008b). Male-derived butterfly anti-

aphrodisiac mediates induced indirect plant defense. *Proceedings of the National Academy of Sciences* **105**: 10033-10038

Gilbert, L.E. (1972) Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proceedings of the National Academy of Sciences of the United States of America* **69**: 1403-1407

Gilbert, L.E. (1976) Postmating female odor in *Heliconius* butterflies: a male-contributed antiaphrodisiac? *Science* **193**: 419-420

Happ, G.M. (1969) Multiple sex pheromones of the mealworm beetle, *Tenebrio molitor* L. *Nature* **222**: 180-181

Hilker, M., Kobs, C., Varama, M. and Schrank, K. (2002) Insect egg deposition induces *Pines sylvestris* to attract egg parasitoids. *The Journal of Experimental Biology* **205**: 455-461

Hilker, M., Meiners, T. (2002a) *Chemoecology of Insect Eggs and Egg Deposition*. Blackwell Publishing

Hilker, M. and Meiners, T. (2002b) Induction of plant responses to oviposition and feeding by herbivorous arthropods: a comparison. *Entomologia Experimentalis et Applicata* **104**: 181-192

Huigens, M.E. (2003) On the evolution of *Wolbachia*-induced parthenogenesis in *Trichogramma* wasps. PhD thesis, Laboratory of Entomology, Wageningen University

Keith, S. and Brown, Jr. (1981) The biology of *Heliconius* and related genera. *Annual Review of Entomology* **26**: 427-456

Leal, W.S., Higuchi, H., Mizutani, N., Nakamori, H., Kasosawa, T. and Ono, M. (1995) Multifunctional communication in *Riportus clavatus* (Heteroptera: Alydidae): conspecific nymphs and egg parasitoid *Ooencyrtus nezarae* use the same adult attractant pheromone as chemical cue. *Journal of Chemical Ecology* **21**: 973-985

Little, D., Gouhier-Darimont, C., Bruessow, F. and Reymond, P. (2007) Oviposition by Pierid butterflies triggers defense responses in *Arabidopsis*. *Plant Physiology* **143**: 784–800

Meiners, T. and Hilker, M. (2000) Induction of plant synomones by oviposition of a phytophagous insect. *Journal of Chemical Ecology* **26**: 221 -232

Naisbit, R.E. (2001) Ecological divergence and speciation in *Heliconius cydno* and *H. melpomene.* PhD thesis, University College London, London

Pashalidou, F. (2008) Hitch-hiking parasitic wasp can associatively learn to exploit butterfly anti-aphrodisiac. MSc thesis, Laboratory of Entomology, Wageningen University

Romeis, J., Babendreier, D., Wäckers and Shanower, T.G. (2005) Habitat and plant specificity of *Trichogramma* egg parasitoids: underlying mechanisms and implications. *Basic and Applied Ecology* **6**: 215-236

Schröder, R., Forstreuter, M. and Hilker, M. (2005) A plant notices insect egg deposition and changes its rate of photosynthesis. *Plant Physiology* **138**: 470–477

Schulz, S., Estrada, C., Yildizhan, S., Boppré, M. and Gilbert, L.E. (2008) An antiaphorodisiac in *Heliconius melpomene* butterflies. *Journal of Chemical Ecology* **34**: 82-93

Shorey, H.H. (1973) Behavioral responses to insect pheromones. *Annual Review of Entomology* **18**: 349-380

Simpson, L. (2007) *Trichogramma* wasps of Kauai: A survey of *Trichogramma* species and the presence of *Wolbachia*. MSc thesis, Laboratory of Entomology, Wageningen University

Stouthamer, R., Hu, J., Van Kan, F.J.P.M., Platner, G.R. and Pinto, J.D. (1999) The utility of internally transcribed spacer 2 DNA sequences of the nuclear ribosomal gene for distinguishing sibling species of *Trichogramma*. *BioControl* **43**: 421-440

Turlings, T.C.J., Tumlinson, J.H. and Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250**: 1251-1253

Vet, L.E.M. and Dicke, M. (1992) Ecology of infochemical by natural enemies in a tritrophic context use. *Annual Review of Entomology* **37**: 141-172

Woelke, J. (2008) Hitch-hiking behaviour of *Trichogramma* wasps on cabbage white butterflies and moths. MSc thesis, Laboratory of Entomology, Wageningen University

Appendix 1

Species/genus	Number	Sequence
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