

Mechanisms behind the Usurpation of *Thyrinteina leucocerae* by *Glyptapanteles* sp.

Introduction

It has frequently been proposed that parasites purposefully manipulate their hosts in order to increase their fitness, usually to the detriment of the host (Lefevre *et al.* 2008, Poulin & Thomas 1999). A recent hypothesis known as the ‘usurpation hypothesis’ argues that parasites manipulate hosts in such a way that the host guards their larvae from hyperparasitoids, or predators of the parasitoids (Harvey *et al.* 2008). Examples demonstrating the usurpation hypothesis are limited, but a few compelling instances do exist. In one system, parasitoid wasps cause aphid hosts to mummify in more concealed sites in order to protect diapausing aphid larva (Brodeur & McNeil 1989). The parasitoid *Hymenoepimecis* sp. has also been shown to induce its spider host *Plesiometa argyra* to build a specialized web designed to carry developing parasitoid cocoons (Eberhard 2000). It appears that the wasps may utilize some kind of fast acting chemical, as removal of the wasp larva results in spiders reverting back to normal web construction (Eberhard 2001). While these examples are compelling, evidence for the specific mechanisms utilized by parasitoids to alter their host’s behavior is largely unknown. However, a recent system of study may provide interesting insight on the mechanisms behind host usurpation.

In this system parasitic wasps lay their eggs in the larva of caterpillars until they are ready to egress from the caterpillar and pupate (Grosman *et al.* 2008). Following parasite egression the host caterpillars cease to feed and walk, and defend the parasitoid pupae by producing head swings against approaching predators (Grosman *et al.* 2008). Caterpillars also often spin a silk web over the parasitoid cocoons in addition to guarding the cocoons (Harvey *et al.* 2008). Following parasitoid hatching the caterpillars die, never turning into moths to

complete their life cycle (Janssen *et al.* 2009). Several studies have shown that the presence of a guarding caterpillar and web increases parasitoid survival rate, and a few have offered insight on the possible mechanisms behind host manipulation (Grosman *et al.* 2008, Harvey *et al.* 2008, Tanaka & Ohsaki 2009).

One compelling explanation comes from a recent study on the caterpillar *Thyrinteina leucocerae* parasitized by the wasp *Glyptapanteles sp.* (Grosman *et al.* 2008). In this study it was found that 1-2 active parasitoid larvae stay behind in *T. leucocerae* hosts after parasitoid egression (Grosman *et al.* 2008). Grosman *et al.* (2008) hypothesized that these larvae stay behind in order to inflict host behavioral changes. This may be the case as similar systems have been found in liver fluke parasitoids that infect ants (Grosman *et al.* 2008). Once inside an infected ant one parasitoid larvae migrates to the host's ganglion and attaches itself, becoming a so called 'brain worm' (Wilson 1977). This brain worm causes ants to attach to blades of grass, increasing their digestion by grazing livestock and thereby increasing parasitic transmission to their definitive livestock host (Carney 1969). This brain worm also loses its ability to infect the livestock host, and thus appears to sacrifice itself to allow for its fellow larvae to succeed (Wilson 1977). It thus seems possible that the *Glyptapanteles sp.* that stay behind in host caterpillars may act in a similar fashion to these 'brain worms'. If this is the case then the post-egression larvae would seem to be exhibiting true altruism, in which they sacrifice their own fitness for the fitness of others (Axelrod & Hamilton 1981). However, it first needs to be shown that these post-emergence larvae are responsible for host behavioral changes and that they do not pupate later on.

A few studies on wasp-caterpillar systems have also shown that parasitoid larvae can interfere with host hormone and neurotransmitter levels (Miles & Booker 2000). One example

comes from the wasp *Cotesia congregata* that parasitizes the caterpillar *Manduca sexta* (Miles & Booker 2000). It was found that coincident with host behavioral changes there was an increase in host octopamine levels, a known neurotransmitter that can interfere with the frontal ganglion of insects (Miles & Booker 2000). These elevated octopamine levels led to decreased caterpillar activity level, and caused the caterpillar's ability to digest food to decrease (Miles & Booker 2000). Given that *T. leucocerae* cease to feed and walk upon parasitoid egression, it is possible that their change in behavior is due to increased octopamine levels.

The aim of this study is to determine whether the *Glyptapanteles sp.* parasitoids which stay behind in *T. leucocerae* are responsible for altering host behavior, and if so what mechanisms they utilize. In order to determine this, laboratory tests will be run looking at the behavior of infected caterpillars as compared to caterpillars in which post-emergence larvae have been removed. If the larvae that stay behind are the cause, then it is predicted that host behavioral alterations will not be seen in caterpillars from which these larvae have been removed. In order to determine a possible mechanism utilized by these post-emergence larvae to alter host behavior, octopamine levels will be measured at various stages in infected and healthy hosts. If the larvae which remain affect octopamine levels to induce host behavioral changes, then octopamine levels should increase following parasitoid egression as this is when host behavioral changes occur.

Experimental Design

Host and Parasitoid Collection & Rearing

T. Leucocerae and *Glyptapanteles sp.* will be collected and reared as in Grosman *et al.* (2008). Caterpillars and parasitoids will be collected from guava trees on the Federal University of Vicosa campus in Brazil, their native habitat. Collected caterpillars will be reared on small

guava trees in cages outside, and will be allowed to mate upon turning into moths. Their eggs will be collected from the cages once per week and will be placed into another enclosed cage to mature.

Adult parasitoids will be allowed to mate in a tube containing a leaf from a guava tree, and then will be placed into a cup containing 8 unparasitized caterpillars for 24h. Once caterpillars are infected and the parasitoids emerge, they will be incubated until adult emergence.

Test 1: Behavioral Changes & Parasitoid Presence

In order to determine whether the parasitoid larvae that stay behind in *T. Leucocerae* are the cause of host behavioral changes, laboratory tests will be run looking at the behavior of infected caterpillars as compared to caterpillars in which post-emergence larvae have been surgically removed. To compare changes in host behavior, the guarding intensity of hosts will be assessed after the egression of parasitoid pupae and any surgical procedures. Observations of whether hosts spin protective silk webs around parasitoid larvae will be noted, and whether caterpillars eat more or less and appear to move more or less will also be recorded before and after parasitoid egression.

For each treatment 30 caterpillars will be utilized. Treatment one will serve as a control, and the guarding behavior of parasitized caterpillars with their post-emergence larvae intact will be measured after parasitoid egression. Treatment two will utilize parasitized caterpillars whose parasitoids will be allowed to naturally egress, and then whose remaining post-egression parasitoids will immediately be surgically removed. The guarding behavior of treatment two caterpillars will be measured after both parasitoid egression and the removal of post-egression parasitoids has occurred. A third treatment will also be run in an effort to account for possible side-effects of surgery. Treatment three will utilize parasitized caterpillars whose parasitoids will

be allowed to naturally egress, and then whose abdomens will be opened up to mimic a surgical removal of remaining parasites. The guarding behavior of treatment two caterpillars will be measured after both parasitoid egression and the fake removal of post-egression parasitoids has occurred.

The intensity of host guarding behavior will be measured 5 days after parasitoid egression and surgical procedures to allow for any behavioral changes of hosts to fully manifest. The intensity of guarding behavior will be measured as in Grosman *et al.* (2008). For each trial a caterpillar and the parasitoid cocoons that had egressed from it will be placed into a 20cm diameter petri dish. A starved stink bug predator, *Supputius cincticeps*, will then be introduced and the intensity of caterpillar guarding behavior will be observed. Observations will be taken for 5min, and the number of head swings by the caterpillar directed towards the predator will be recorded as a measure of guarding intensity.

Before performing surgical procedures in treatments two and three, caterpillars will be anesthetized with CO₂ and tools will be disinfected as in Miles & Booker (2000). Caterpillars will be placed into a chamber with a constant CO₂ supply to their trachea, and their abdomens will be left exposed. All surgical tools will be placed into a solution of 95% ethanol before use, and the abdomens of each caterpillar will be cleaned with ethanol prior to incision. After the careful removal of post-emergence larvae, all surgical incisions will be sealed with dermatological glue as in Miles and Booker (2000). However, any ruptures in the abdomen due to the prior egression of parasitoid larvae will remain open. For treatment two the abdomens of caterpillar hosts will be opened and then sealed with no removal of post-egression parasitoids.

Infected caterpillars are known to guard parasitic cocoons, spin protective silk webs over parasitic cocoons, and move and eat less (Grosman *et al.* 2008, Harvey *et al.* 2008). Therefore, if

the parasitoids which stay behind in *T. leucocerae* are responsible for altering host behavior, then caterpillar guarding behavior should be less intense or absent in treatment two since the post-emergence larvae will have been removed. The caterpillars in treatment two should also not spin webs over parasitic cocoons, and they should eat and move more often than caterpillars from which the post-emergence larvae are intact. However, if the post-emergence larvae are not responsible for host behavioral changes, then hosts from which they have been removed should exhibit equal changes in behavior to those caterpillars whose larvae remain intact.

Test 2: Control Mechanism Behind Manipulation

As increased octopamine levels are known to cause decreased activity and feeding behavior in other infected caterpillar species, the behavioral changes in *T. Leucocerae* may be due to the same mechanism (Miles & Booker 2000). In order to determine this, octopamine levels will be measured in three treatment groups similar to Adamo *et al.* (1997). In treatment one the octopamine levels of infected caterpillars will be measured before parasitoid emergence, at the time of parasitoid emergence, and 5 days after parasitoid emergence. As a control, uninfected caterpillar octopamine levels in treatment two will be measured on the same days as the infected caterpillars. As a control for the decrease of food intake in post-emergence caterpillars, a set of uninfected hosts in treatment three will also be starved for a day and then tested for octopamine levels. A total of 30 caterpillars will be utilized for each treatment.

As octopamine is found in the haemolymph of caterpillars, haemolymph samples will be drawn by clipping the horns of *T. Leucocerae* and using a 25 μ l Hamilton syringe to collect the emerging fluid. A total of 10 μ l will be drawn from each caterpillar at each stage of sampling. Each sample of 10 μ l will then be added to 40 μ l of perchloric acid, and then spun in a centrifuge for 5min at 4 °C. The supernatant will then be added to 60 μ l of HPLC-water and placed into an

outsampler tube. Then chromatographic separation of octopamine will be conducted using a Vydac C-18 HS-54–15 HPLC column. Identification of octopamine will be based on retention times as compared to standards, and peak retentions will be measured and converted to pg/ μ l haemolymph. These sampling methods are as in Adamo *et al.* (1997).

If the behavioral changes seen in *T. Leucocerae* are caused by octopamine levels, then an increase in octopamine should be seen in samples drawn from infected hosts after parasitoid egression. This is because caterpillar hosts only begin showing behavioral changes after *Glyptapanteles sp.* has emerged (Harvey *et al.* 2008). On the contrary, the control treatments utilizing uninfected hosts should show no change in octopamine levels if these levels are what cause host behavioral changes. If it is found that octopamine levels increase post-egression, this will suggest that the parasitoids indeed control host behavior through increasing the level of this neurotransmitter.

Conclusion

It is the purpose of this study to determine whether the post-egression larvae in *T. leucocerae* are responsible for altering host behavior, and if so how they go about inflicting these changes. If the larvae which stay behind affect host behavioral changes, this will give interesting insight into the mechanisms behind host manipulation. It will also offer an avenue for future research looking at whether this is a system of true altruism, which would require further studies looking at whether these post-emergence larvae pupate later on or sacrifice themselves.

References

- Adamo SA, Linn CE, Beckage NE. Correlation between changes in host behaviour and octopamine levels in the tobacco hornworm *Manduca sexta* parasitized by the gregarious braconid parasitoid wasp *Cotesia congregata*. *Journal of Experimental Biology* (1977) 200: 117-127.
- Axelrod R., Hamilton WD. The Evolution of Cooperation. *Science* (1981) 211: 1390-1396.
- Brodeur J, McNeil JN. Seasonal microhabitat selection by an endoparasitoid through adaptive modification of host behavior. *Science* (1989) 244: 226-228.
- Carney WP. Behavioral and Morphological Changes in Carpenter Ants Harboring Dicrocoeliid Metacercariae (1969) 82: 605-611.
- Eberhard WG. Spider manipulation by a wasp larva. *Nature* (2000) 406: 255-256.
- Eberhard WG. Under the influence: Webs and building behavior of *Plesiometa argyra* (Araneae, Tetragnathidae) when parasitized by *Hymenoepemcis argyraphaga* (Hymenoptera, Ichneumonidae). *Journal of Arachnology* (2001) 29: 354-366.
- Grosman AH, Janssen A, de Brito EF, Cordeiro EG, Colares F, Fonseca JO, Lima ER, Pallini A, Sabelis MW. Parasitoid increases survival of its pupae by inducing hosts to fight predators. *Plos One* (2008) 3: e2276.
- Harvey JA, Kos M, Nakamatsu Y, Tanaka T, Dicke M, Vet LEM, Brodeur J, Bezemer TM. Do parasitized caterpillars protect their parasitoids from hyperparasitoids? A test of the 'usurpation hypothesis'. *Animal Behaviour* (2008) 76: 701-708.
- Janssen A, Grosman A, Cordeiro EG, de Brito E, Fonseca JO, Colares F, Pallini A, Lima ER, Sabelis MW. Context-dependent fitness effects of behavioral manipulation by a parasitoid. *Behavioral Ecology* (2009) 21: 33-36.
- Kester KM, Jackson DM. When good bugs go bad: intraguild parasitism predation by *Jalysus wickhami* on the parasitoid, *Cotesia congregata*. *Entomologia Experimentalis et Applicata* (1996) 81: 271-276.
- Lefevre T, Roche B, Poulin R, Hurd H, Renaud F. Exploiting host compensatory responses: the 'must' of manipulation? *Trends in Parasitology* (2008) 24: 435-439.
- Miles CI, Booker R. Octopamine Mimics the Effects of Parasitism on the Foregut of the Tobacco Hornworm *Manduca Sexta*. *Journal of Experimental Biology* (2000) 203: 1689-1700.

Poulin R, Thomas F. Phenotypic variability induced by parasites: Extent and evolutionary implications. *Parasitology Today* (1999) 15: 28-32.

Preti, A. Suicide among animals: A review of Evidence. *Physiological Reports* (2007) 101: 831-848.

Tanaka S, Ohsaki N. Does manipulation by the parasitoid wasp *Cotesia glomerata* (L.) cause attachment behaviour of host caterpillars on cocoon clusters? *Ethology* (2009) 115: 781-789.

Wilson, DS. How Nepotistic Is the Brain Worm? *Behavioral Ecology and Sociobiology* (1977) 2: 421-425.