Manipulation From Within:
A Review on Behavioral Changes of Insect Hosts by Parasitic Organisms

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Abstract
Manipulation of host behavior by various parasitic organisms is an occurrence that has been observed in several taxa, including vertebrate and non-vertebrate host species. Insects are often used as intermediate hosts within a parasitic lifecycle and can be considered a stepping-stone for the parasite in order to reach a definitive host and complete its lifecycle. Intermediate host behavior manipulation in insects can be highly beneficial for the parasite itself. Parasites cannot complete their lifecycle and successfully reproduce without first reaching their definitive host. Which is why it is not uncommon to observe changes in host behavior that would increase the chances of the intermediate host (and ultimately the parasite) encountering the next sequential host in the lifecycle. These behavioral modifications can be observed as abnormal behaviors that the insect would not normally perform, being that the consequences of said behavior often result in the insect being more easily spotted by a predator and eaten. Insects are often vectors for many parasites that can cause health problems and diseases in people and livestock around the world. To further understand the behavioral modifications of these insects by the parasites they carry can lead to possible control or eradication of certain vector borne pathogens and parasites. This review article will summarize recent research on this particular field of study and convey the current knowledge of insect host behavioral modification by common fungal, prokaryotic, and eukaryotic parasites.
**Introduction**

Host-parasite relationships are complex and vary widely in the interactions between the many species that play a role in this intimate pairing of organisms. As if invading the host body and robbing it of its energetic resources is not enough, many parasites take it one step further and influence the behavior of their inhabited host. This evolutionary trait of parasites has demonstrated its effectiveness by its prevalence between a wide array of parasitic species and the impacted host counterparts.

However, insects in particular tend to stand out as model host species for studying this behavioral modification. Not only are they important components in the lifecycles of parasites, usually as intermediate hosts, but they also tend to be vectors of many harmful diseases and pathogens that effect people around the world. This demonstrates the value in studying the interactions among parasites and their insect hosts, specifically their capabilities of behavioral modification.

At one point in time this ability of parasites to alter their hosts’ behavior was unheard of. It was a relatively new discovery with much fascination behind it. An example of an early study in this field was done on *Periplaneta americana*, a cockroach intermediate host of the archiacanthocephalan, *Moniliformis moniliformis*. This research demonstrated a change in predator avoidance behaviors by the host cockroach when infected with *M. moniliformis*, which increased its vulnerability, thereby making successful transmission of the parasite more likely to occur (Moore, 1983).
These early studies laid the foundation from which present day research is beginning to progress beyond mere demonstration of behavioral changes in hosts and into learning the mechanisms behind this perplexing action. This review article is intended to discuss the current efforts of research on this subject and give an overview of recent discoveries on the underlying mechanisms of behavior modification in insects by various parasitic organisms. The topics of discussion will cover how behavioral changes in the host are influenced by genetics, different molecular effects on the central nervous system, and responses in the host immune system; as well as manipulation of the definitive host to induce behavioral changes in the insect and alternative explanations for behavioral modifications within the host-parasite system.

**1. Genetic Influences**

Being that genetics are one of the most fundamental components of any biological system, it is important to understand the role of genetics in parasitic behavioral modification. Genetics also play a substantial role in the social structure and evolutionary development of eusocial insects, such as honeybees and bumble bees. This basis highlights the importance of understanding how parasites can influence the behavior of critical pollinators from an ecological standpoint.

In a study done on hybrid *Apis mellifera mellifera* and *A.m. ligustica* honeybees, researchers tested the comparative effects on foraging behavior when bees were separately infected with two kinds of parasites: *Varroa destructor*, which is an ectoparasitic mite, and *Nosema ceranae* which is an obligatory, intracellular spore-forming fungus of honey bees. While they were unable to observe any
differences in social interactions or behaviors of healthy versus infected bees, they were able to demonstrate a difference in the cuticular hydrocarbon (CHC) profiles when bees were infected with either parasite. For this particular study, they propose that lack of aggression towards infected bees by other members of the hive to promote removal of sick bees (which had been shown in previous studies) could possibly be because infected individuals are distinguished by their difference in CHC profiles, suggesting that they leave willingly as a form of social altruism in order to prevent further loss of individuals. Ultimately they were able to compare brain transcriptional profiles of the bees infected with the two parasites and found that these bees shared more common gene changes than with other non-infected bees. They suggest the possibility of these gene changes in parasitized bees as being the driving force that causes voluntary departure of the hive when infected. Other possible explanations are discussed as well, with suggestions on future work to better understand these findings (McDonnell, et. al., 2013).

Another study that looked into the genetic component of host-parasite interactions compared three bumblebee species: Bombus bimaculatus, B. griseocollis, and B. impatiens. In this study they test to see whether or not Physoscephala tibialis, a conopid parasitoid fly, can induce self-burial in all three bumblebee host species after laying its eggs inside them. However, they discuss that these adaptations of the conopid fly to manipulate host behavior do not directly impact the fitness of the bumblebee simply because the parasitoid is only influencing location of host death. Yet this manipulation enhances the fitness of the parasitoid fly by allowing larvae and pupae within the bee to occur in an environment that is better protected from
predation and overwintering during development. Their results showed that the parasitoid fly could induce self-burial in all three species. However, this behavior was only performed at a frequency of 17.4% in *B. griseocollis*, but at around 70% for both *B. bimbaculatus* and *B. impatiens*. The two species that had a much higher and similar frequency of self-burial behavior are also more closely related to each other than to *B. griseocollis*. The authors suggest that due to the fitness of the host not being directly affected, there is limited selection involved that would result in bumblebees resisting this behavioral manipulation, while it is still strongly selected for in the parasitoid fly. They say a possible consequence of this might be specialization of the parasitoid fly to certain bumblebee species given that they might express variation in behavior when parasitized. This would be consistent with the results they generated. While this study provides further understanding of this particular parasitoid-host interaction, they mention that further studies need to be done in order to comprehend the mechanisms at work (Malfi, et.al. 2014).

**II. Molecules Effecting Central Nervous System**

As the paper in the previous section has demonstrated, parasitoids can induce drastically abnormal behavior responses to direct what they want the host to do and where they want the host to be. However this type of behavioral manipulation is not limited to parasitoids. It can also be implemented in hosts infected with other parasites. Research on this highly manipulative strategy has lead to increased realization of chemical signaling within the central nervous system (CNS) of the host and its mechanistic role.
In a study on *Paragordius tricuspidatus*, a common parasite of orthopteran insects, proteomics were used as a biological assay to link the understood concepts of “genome sequence and cellular behavior” (Biron, 2006) in the host cricket species *Nemobius sylvetris*. Hairworms are unique in that the adult stage is free-living while the juvenile stage requires a host for development. When the hairworm is fully developed and is ready to emerge, it manipulates the behavior of its host by directing it to a water source, which is a necessary environment for its continued development and survival. Proteins within the head of infected orthopterans demonstrated differential expression with the highest values correlating to periods when the host was undergoing behavioral alterations. These changes in expression are speculated to be a result of indirect modification of the host genome by the parasite. This study also demonstrated that the hairworms produced Wnt molecules mimicking those of the cricket, thereby disrupting normal CNS functions. The researchers suggest it is likely these Wnt molecules are put directly into the host CNS via direct contact with the brain by physical contact with the parasite. While there are many components of these processes that remain unclear, this study has helped develop a framework of useful information from which continued research can branch (Biron, et.al. 2006).

In another study that explored molecular mechanisms of host-parasite interactions, researchers surveyed metabolites of *Ophiocordyceps unilateralis sensu lato* (s.l.), a parasitic fungus that promotes spore-dispersal by modifying behavior in host ants through manipulation of the CNS. Ants infected with this fungus climb up and latch onto a piece of foliage; they remain in place due to muscle atrophy in the
mandibles. After death occurs, the fungus is able to grow out of the ant and form reproductive structures from which it can begin spore dispersal. Researchers tested for host manipulation in *Camponotus castaneus* and *Camponotus americanus*, which are naturally infected ant species, and in *Camponotus pennsylvanicus* and *Formica dolosa*, which have yet to be found infected in nature even though all of these species are sympatrical. Their results showed that *O. unilateralis* (s.l) was able to infect and kill all four ant species, however host manipulation was absent from the two species that are not naturally infected. Metabolite surveys lead to discovery of candidate compounds that are likely to be involved in manipulation of the host brain. Support for this was demonstrated by the enhanced secretion of guanobutyric acid (GBA) and sphingosine from the fungus when grown next to the brains of hosts that are naturally infected. These results demonstrate species-specific interactions among *O. unilateralis* (s.l) and its host species by way of co-evolution with one another. It provides the opportunity for further advancement and research in understanding the mechanism of this and other host-parasite interactions on a molecular level (de Bekker, et.al. 2014).

**III. Impacting Immune Response**

While manipulating the CNS of a host can be an effective method in parasites that depend on their host as a developmental vessel, other approaches to altering host behavior have evolved to better suit alternative forms of parasitism. For example viral pathogens and prokaryotic parasites seem to impact the immune response of their hosts due to the differing nature of their transmission.
There was a study performed on the mosquito *Anopheles stephensi*, a vector of malaria, in which its responsiveness to vertebrate host attraction was tested on a neurophysiological and behavioral level. In their initial experiments they tested mosquitos infected with *Plasmodium yoelii* and observed sensitivity changes in odor receptors of the maxillary palps corresponding to specific stages of the parasite within the mosquito, as well as the proximity of host location. These results were consistent the hypothesis that malarial parasites manipulate the behavior of the insect host. However, they observed mosquitos without signs of infection demonstrating similar responses to mosquitos that were positively infected. In order to further investigate this unexpected occurrence, they activated an immune system response in uninfected mosquitos by microinjecting them with uninfectious heat-treated *Escherichia coli* bacteria. The *E. coli* treated mosquitos showed similar changes in attraction to vertebrate hosts as compared to the malaria-infected mosquitos. Ultimately this result “suggests that while altered mosquito behaviour may be a product of parasite interaction with the immune system, the pathways used are not uniquely stimulated by the parasite” (Cator, et.al. 2013). Researchers of this study acknowledge that an alternative explanation to this immune response may be driven by mosquito host response as an adaptive trait. Other phenotype changes in mosquitos have been proposed as results of malarial manipulation and this study offers new information that can be applied to better understanding causes behind these changes (Cator, et.al. 2013).

In another study regarding host immune response, researchers detected a pathogen in the cricket *Gryllus texensis*, which turned out to be the insect iridovirus,
IIV-6/CrIV. They were able to detect the virus by means of electron microscopy (EM) in that it allowed them to see a high concentration of the virus in the fat bodies (an important organ needed for production of protein, storage of lipids and immune response) in the infected cricket. The identity of the virus was confirmed by analysis of polymerase chain reaction (PCR). Fat bodies of infected individuals became enlarged, production of eggs in females withered leaving them sterile, and although the virus didn’t appear to be detected in the testis of males by EM, sperm from infected males showed reduced motility. Despite their complete lack of fecundity, this did not prevent them from mating. Surprisingly, researchers observed quicker attempts to court females by infected males compared to uninfected males. In fact, another experiment they did demonstrated that the virus is sexually transmitted.

The continuation of mating therefore is important for the survival of the virus. This could possibly explain why there was a lack of immune signals in infected crickets, which would normally induce sickness behavior. If this were indeed a mechanism behind these behavioral changes, it would benefit the virus if the host continued to appear healthy to potential mates as well as continue its own efforts to mate (Adamo, et.al. 2014).

**IV. Manipulation of Definitive Host**

Most of the studies on behavioral modifications focus on the direct and internal influences from the parasite when it inhabits the insect host. However, some studies are starting to take an alternative approach by noting there may also be potential influences the parasite creates when present in the definitive (vertebrate) host that improve its chance of transmission to the insect host.
One study on this topic analyzed *Culex pipiens* mosquito preferences when given the choice of uninfected birds and birds chronically infected with the *Plasmodium relictum* malaria parasite. Microsatellite typing allowed for a quantifiable assessment of the blood ingested by mosquitoes and presented data suggesting that birds chronically infected with malaria were more appealing to mosquitoes. This preference appeared to be the same whether the mosquito was infected or not. This is consistent with supporting the hypothesis that malaria parasites increase their transmission success by making the avian host more attractive. However, this study also attempted to analyze the biting behavior of infected and uninfected mosquitoes to see if this behavior was manipulated by *P. relictum* but the results did not demonstrate any significance. They suggest further studies to improve the understanding of the underlying mechanisms created by the parasite, vector, and vertebrate host (Cornet, et.al. 2013).

Another study involving the complex species interaction surrounding the malaria pathogen does indeed provide further insight to some of the queries left unresolved from the previous mentioned study. Researchers investigated how mice infected with *Plasmodium chabaudii* increased in attractiveness to mosquitoes compared to healthy uninfected individuals. They found that mice infected with malaria are attractive to mosquitoes during a critical period when the transmissible gametocyte stage is present in high levels within the mice. This period of increased attraction corresponded to an observable rise in volatile emissions from the infected mice. The study suggests that the malaria parasite manipulates the behavior of the mosquito vector by using the same odors of healthy mice and exaggerating them the
infected mice, making them more enticing, thereby increasing transmission potential. Upon further investigation of the volatile blends seen at different stages in infected mice, there is more blending of healthy and infected volatile components during the post-chronic phase as compared to the acute and chronic phases where there appears to be more of a distinction between the healthy and infected odors. This study is of particular importance not only because it provides great detail about the mechanistic manipulations of the insect vector via its vertebrate host, but it also has the potential to be a useful diagnostic tool for individuals infected with malaria due to their distinct odor changes (De Moraes, et.al. 2014).

**V. Alternative Explanations for Behavioral Modification**

Although this topic has been mentioned in passing while discussing some of the other studies covered within this review, certain studies have demonstrated data more indicative to alternate explanations of host behavioral modification, other than merely benefitting the parasite.

Recall that *N. ceranae* is an obligatory fungus of honeybees. Another study was done on its apparent effects concerning *A. mellifera* worker bees, which focused on modification in their flight activity and the reasoning behind this change in behavior. In this study, an optic bee counter was used to compare how flight activity differed between infected and healthy bees. Infected bees demonstrated premature flight activity as well as increased flight activity compared to uninfected bees. They also tested the levels of Ethyl Oleate (EO) a pheromone linked to foraging behavior in workers. They found that EO levels were higher in infected bees, which correlates with the observed increase in activity and decrease in life span. This increased level
of EO in infected bees might possibly prevent behavioral maturation in healthy bees of the same age, thereby explaining the decreased level of activity. They suggest that this might be a possible mechanism to help protect the hive from further infection and loss of individuals. The infected bees perform the more challenging/risky task of foraging since their fitness is already compromised, while the healthy bees’ lifespans are extended due to their involvement in easier, safer tasks within the hive (Dussaubat, et.al. 2013).

The last article of discussion concerns malaria yet again but with a different set of reasons explaining the behavior changes of the vector. This study investigates *Anopheles gambiae* mosquitos’ attraction to nectar sources when infected with *Plasmodium falciparum*. They found that mosquitos containing the oocyst stage of *P. falciparum* had an increase in attraction to plant odors by 30% and an increase in probing activity by 77%. As for mosquitos infected with the sporozoite stage, attraction increased by 24% and probing increased by 80%. Increased uptake of sugar appears to occur at the oocyst stage and decreases once in the sporozoite stage. They discuss multiple possibilities involved in the change of behavior of *A. gambiae* regarding nectar sources. It might be due to physiological changes implemented by the mosquito to counteract the infection of malaria, or it may be due to manipulation by the parasite to increase transmission success. They suggest further testing to amplify the understanding of this host-parasite interaction so as to increase possibilities of disease control on deadly pathogens such as malaria (Nyasembe, et.al. 2014).
This paper also emphasizes the overarching theme demonstrated in any and all host-parasite relationships, which is the never-ending arms race against one another. This concept is not a simple model of one organism influencing the other. It is a constant battle between two organisms attempting to gain advantage over the other and yet both are fighting for the same thing: survival.

Reference List


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