Manipulative wasps

Parasite manipulation of host behavior by parasitoid wasps

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Summary

Parasite-host interactions are part of complex symbiotic relationships that often precede millions of years of coevolution. One specific parasitic strategy commonly found in parasitoids is parasite manipulation. Parasite manipulation is the manipulation of the host by the parasite that is purposefully induced and results in the increased fitness of the parasite. This thesis explores our current knowledge on the mechanisms of parasite manipulation of host behavior by parasitoid wasps. The two main pathways targeted in parasitoid manipulation are the endocrine and neural pathways. Both endocrine and neural systems are responsible for behavioral responses in the host, and alterations in these pathways can result in behavioral manipulation. In many cases, the immune system is targeted through these pathways, which can directly result in behavioral responses. The mechanisms by which these systems are affected can be caused directly by the parasitoid wasp but also by symbionts. Venom injection and the secretion of chemical compounds by the larvae are two mechanisms directly employed by the parasitoid wasps. So far, symbiotic Polydnaviruses and RNA viruses have been associated with parasitoid manipulation by parasitoid wasps. These viruses likely instigate changes in gene regulation and transcription in the host. Two case studies demonstrate that parasitoid manipulation can be extremely complex, and parasitoids often utilize multiple mechanisms and pathways to manipulate their host. As of now, there has been little research on the exact mechanisms of host manipulation by parasitoid wasps. Gaining a broad understanding of these mechanisms is significant for our evolutionary knowledge of parasitism and is also ecologically and commercially relevant, as parasitoid wasps are often used as biological control agents. It is therefore important that future research focuses on further exploring the proximate mechanisms of parasite manipulation by parasitoid wasps.

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Introduction

Symbiotic relations have been a long-term subject of study in biology, with symbiosis first described by de Bary (1879). Symbiosis includes all interactions where two different organisms live in close association with each other (de Bary, 1879). These interactions are classified in three main categories: mutualism, commensalism and parasitism. These categories differ based on the effects they have on the organisms involved. Parasitism is a symbiotic relationship where one organism directly benefits at the expense of its symbiotic partner (Noble et al., 1982). Parasites are either endoparasitic, living inside the body of the host, or ectoparasitic, living on the surface of the host's body (Bush, 2001). Parasitism has led to a co-evolutionary arms race between hosts and their parasites, this evolutionary pressure resulted in various strategies of parasitism (Buckingham & Ashby, 2022; Poulin, 2011).

There are six major strategies of parasitism (Poulin, 2011), one of which is parasitoidism. This specific strategy is distinguished by the host-parasite interaction leading to the inevitable death of the host (Poulin, 2011; Poulin & Randhawa, 2015). Parasitoidism therefore looks very similar to predation. The main differences are that parasitoids most of the times feed on live tissues, whereas predators consume prey that has been killed or is killed during consumption (Johnson, 2013). Addtionally, parasitoids require a single organism to develop, while predators have to feed on multiple prey organisms during development (Johnson, 2013). Parasitoids develop inside the host, but during their adult phase, they are free-living (Johnson, 2013). Free-living parasitoid wasps primarily feed on nectar and pollen of flowers (Lee & Heimpel, 2008). Several taxa of the insect superorder Endopterygota include parasitoids organisms, with most being parasitoids wasps (Polaszek & Vilhemsen, 2023). Parasitoid wasps are highly specialized, and species often parasitize only a relatively small clade of insects (Strand & Obrycki, 1996).

Parasitoid strategies are diverse and not mutually exclusive (Polaszek & Vilhemsen, 2023). The broad spectrum of parasitoid strategies is a sign of extensive coevolution between parasitoids and their hosts. Each specific strategy is related to the wasps ecological niches (Polaszek & Vilhemsen, 2023). Parasitoids that parasitize other parasites are known as hyperparasitoids. Kleptoparasitoidism is a form of parasitoidism where the food reserves of the host are targeted. These reserves are often used for reproduction and can also include the targeting of the host's eggs. Phoresy is a strategy where one species uses another species for transportation. Parasitoids can also be classified as gregarious, where multiple offspring develop in a single host, or solitary, where one offspring develops from a single host. Additionally, there are two strategies that refer to the way the host is consumed. Koinobionts are parasitoids that allow their hosts to continue developing after infection, until they are eventually consumed or killed. Idiobionts are parasitoids that kill or paralyze the host and immediately consume them. In most cases, koinobionts are endoparasites and idiobionts are ectoparasites.

Some parasites have the ability to manipulate their hosts, a phenomenon called parasite manipulation (Perrot-Minnot & Cézilly, 2010; Poulin, 2010; Weinersmith, 2019). This parasitic strategy is common across many parasites and includes any alteration in behavior, physiology or morphology of the host that is purposefully caused by the parasite and improves the fitness of the parasite (Poulin, 1994). The most well-known example is the fungus *Ophiocordyceps unilateralis*, commonly known as the zombie-ant fungus, which parasitizes and manipulates formicine ants (Evans et al., 2011). Infected ants climb vegetation and eventually die while biting into the vegetation to hold their position, a behavior induced by the fungus which facilitates the spread of it spores by releasing them from a height (Andersen et al., 2009). This behavior results in an increased fitness due to higher transmission rates of the fungus (Andersen et al., 2009). The fungus releases chemicals in the ant that influences the neurochemistry resulting in the behavioral changes (de Bekker et al., 2021).

One of the most well-studied groups of animals on parasite manipulation are parasitoid wasps. Millions of years of coevolution between these wasps and their hosts have resulted in the evolution of this parasitic strategy (Polaszek & Vilhemsen, 2023). To gain an evolutionary understanding of parasite manipulation, it is important to research the mechanisms behind these strategies in multiple taxa, especially since this strategy has evolved convergently across these taxa. In recent years, research on the mechanisms used by parasitoid wasps to manipulate their host's behavior has increased. However, there has been little synthesis of reviews on the mechanisms of manipulation by these wasps. This report explores the broad variation in proximate mechanisms of parasite manipulation by parasitoid wasps, with a specific focus on behavioral changes.

The research question is: What are the proximate mechanisms of parasite manipulation of the host behavior by parasitoid wasps? To answer this question, literature on mechanisms of parasite manipulation is reviewed. The different aspects of parasite manipulation are evaluated throughout the report and supported by several case studies.

This study aims to provide an overview of our current knowledge on parasite manipulation by parasitoid wasps and to identify the gaps in existing research while also gaining a better understanding of parasite manipulation and the effects of parasitoids on their hosts. Getting a better understanding of parasite manipulation by parasitoid wasps is not only evolutionary relevant, but also ecologically, as parasitoid wasps are commonly used as biological control agents (Wang et al., 2019). It is important to understand the ecological impact of these parasitoid wasps for possible implications in biological control strategies.

Results

Pathways of behavioral manipulation

Endocrine and neural systems are often directly related to the behavior of an organism (Harris-Warrick & Marder, 1991; Nelson, 2010). Manipulation of host behavior by parasitoid wasps is generally caused by changes in either neurobiological or endocrinological mechanisms (Adamo et al., 2016; Arvidson et al., 2019; Kloss et al., 2017). In these systems alterations can be induced by compounds that work on the hosts hormonal balance and signaling pathways or neural systems. These compounds can disturb the natural balance of the compounds in the host, or they can be introduced as foreign compounds. Changes in the neuromodulatory or neurotransmitter pathways can directly interfere with the processing of information by the host and in turn the response to their environment (Libersat et al., 2018). Changes in endocrinological systems can disrupt the hormonal balance, leading to various physiological and behavioral consequences and disturbing hormonal signaling pathways (Lafferty & Shaw, 2013).

Alterations in both endocrine and neural systems can affect the immune system of the host (Dantzer, 2018; Stelzer & Arck, 2016). In many parasitoid cases the immune system of the host is targeted to protect the offspring from immune responses that can lead to their death (Weinersmith, 2019). The behavioral immunity is an important part of the hosts immunity against parasites (de Roode & Lefèvre, 2012). The manipulation of behavioral immune responses can benefit the offspring in more ways than just protection against the host. Multiple parasitoid wasps induce a response in their host known as direct bodyguard manipulation (Harvey et al., 2013). Direct bodyguard manipulation alters behavioral immune responses, causing the host to protect the offspring from predators or hyperparasitoids. This form of manipulation has so far been observed in two families, Braconidae and Ichneumonidae (Harvey et al., 2013; Mohan & Sinu, 2022). Some other behaviors that are affected by parasite manipulation are feeding, grooming, locomotion and reproduction (Lafferty & Shaw, 2013; Moreau & Asgari, 2015).

Mechanisms of introduction and alteration of chemical compounds

Wasp mediated mechanisms

There are multiple ways that parasitoid wasps use to alter neural or endocrine systems of the host, Table 1 presents an overview of the pathways previously mentioned and the mechanisms addressed here. Many parasitoid wasp species inject venom into their host (Haspel et al., 2003). This venom can contain chemical compounds that are similar to or identical to the host's hormones or neurotransmitters. The venom of most ectoparasitoids contains substances that induce lethargy or paralysis in the host, but there is also evidence for the venom affecting the immune system or causing developmental arrest in the host (Moreau & Asgari, 2015). In endoparasitoids the injection of venom usually does not lead to long-term lethargy or paralysis and the venom of these parasitoids is focused on regulating development, metabolism and immune responses (Moreau & Asgari, 2015).

Another mechanism that is used by parasitoid wasps is the secretion of compounds into the body of the host. Unlike venom injection that is performed by the adult parasitoid wasp, the secretion of these compounds is done by the offspring of the adult wasp (Adamo et al., 2016). An example is the effect of larval saliva from the *Pteromalus puparum*. Shi et al. (2022) found that the saliva that is secreted by the larvae from this endoparasitic parasitoid wasp suppresses the host immunity during their development. Unlike the injection of venom which takes place on the single occasion of infection, the larvae can control internal mechanisms of the host during the developmental period and it is suggested that the manipulation can change during the development of the larvae (Kloss et al., 2017; Shi et al., 2022). Multiple parasitoid wasps of the Polysphincta genus, which parasitize on spiders, induce a behavioral response in their host related to molting, but only during the final stages of larval development (Kloss et al., 2017).

Both previously mentioned mechanisms involve direct introduction of neural or endocrine compounds by the parasite into the hosts. There are also mechanisms involving indirect interactions between the parasitoid and its host, which alter the regulation of genes and transcription in the host cells and are often mediated by symbionts (Beckage & Drezen, 2011).

Symbiont mediated mechanisms

Some parasitoid wasps use symbiotic relationships in order to manipulate their host and increase their own fitness. This is manipulation is done through microbial symbionts, symbionts that have been found in parasitoid wasps and their hosts include DNA viruses, Polydnaviruses and RNA viruses (Beckage & Drezen, 2011; Dheilly et al., 2015; Herniou et al., 2013). The mechanisms by which these viruses directly affect the host are not well studied, studies suggest that the main function of these viruses is to alter the immune function of the host (Amaya et al., 2005). This is likely achieved by altering the gene regulation and/or protein transcription inside the host's cells. Repression of immune function prevents the larvae of the parasitoid wasps to be attacked by the immune system of the host.

The symbiotic viruses in parasitoid wasps are endosymbionts, they live inside the wasp and take part in a mutualistic relationship (Beckage & Drezen, 2011). The parasitoid wasps provide an environment for the viruses to replicate and directly helps in transmission of the viruses between organisms. In return the viruses aid the wasps to successfully parasitize their hosts, resulting in an increased fitness in both the wasp and virus.

Polydnaviruses have a long history of coevolution with many parasitoid wasps species, with some viruses originating from virus evolution over a time period of 100 million years (Herniou et al., 2013). Polydnaviruses are directly integrated into the genome of the wasps and are largely produced in the ovaries of the parasitoid wasps, ensuring direct transmission of the viruses into the offspring (Herniou et al., 2013). In the polydnaviridae family, there are two distinct genera, the Bracovirus and Ichnovirus, which are associated with the wasp families Braconidae and Ichneumonidae (Herniou et al., 2013). The wasps of these families are koinobionts and certain subfamilies that parasitize on lepidopteran larvae have been associated with Polydnavirus symbionts. An example of one of these wasps that uses Polydnaviruses is the *Cotesia congregata* that parasitizes on caterpillars of the *Manducta sexta* (Amaya et al., 2005*)*. It is believed that the Polydnavirus present in these wasps suppresses the hosts immune system.

Beneficial RNA and DNA viruses have also been discovered in these wasp families (Beckage & Drezen, 2011). Not all DNA and RNA viruses present in these wasps affect the host of parasitoid wasps, there are also viruses that increase the fitness of the parasitoids by altering their life-history, like in the *Pteromalus puparum* (Coffman et al., 2020). For the first time a study on parasite manipulation by the *Dinocampus coccinellae* provided evidence of behavioral manipulation that was directly caused by RNA virus infection (Dheilly et al., 2015). Dheilly et al. (2015) showed that the replication of the RNA virus in the host's nervous tissue resulted in an antiviral response and neuropathy that likely caused the behavioral response in the host.

Manipulation by	Wasp		Symbiont (RNA-, Polydnaviruses)
Mechanism	Injection	Secretion	Transcription/ Gene regulation
Pathways	Endocrine, Neural	Endocrine, Neural	Endocrine, Neural
Target	Immune system, Movement, Development, Metabolism	Immune system, Movement, Development, Metabolism	Immune system
Parasitoid strategy	Idiobiont, Koinobiont	Idiobiont, mostly Koinobiont	Idiobiont, mostly Koinobiont

Table 1. Summary of mechanisms and pathways of parasitoid behavioral manipulation by parasitoid wasps and their symbionts. The table also includes the main targets of the mechanisms and pathways and their associated parasitoid strategies (idiobiont or koinobiont).

Physical agitation and energy drain

Some parasites induce a behavioral response in their host that is not considered parasite manipulation. It is important to note that the definition of parasite manipulation by Poulin (1994) specifically mentions that the behavioral alterations must be purposefully induced by the parasite. Two mechanisms that can result in behavioral changes that are sometimes misinterpreted as parasite manipulation are energy drain and the physical presence of the parasite on or within the host (Lafferty & Shaw, 2013; Weinersmith et al., 2017). Energy drain refer to the depletion of nutrients by the parasite in and/or on the host (Lafferty & Shaw, 2013). This depletion of nutrients can result in a decrease in activity or changes in feeding behavior. The physical presence of parasites can result in agitation of the host, agitation can trigger multiple behavioral responses in the organism. An example of these responses is escape behavior, where the host tries to flee from its current situation or environment, to protect itself against external threats.

Both mechanisms trigger a behavioral response that is already innate to the host. In these cases, the mechanism acts as a stimulus that results in expression of that behavior and are therefore, not necessarily purposefully caused by the parasite. Studies performed on the *Euderus set*, a hyperparasitoid of the *Bassettia pallida* gall wasp have proposed both mechanisms as hypotheses for behavioral changes in the host caused by parasite manipulation (Weinersmith et al., 2017). This is arguably incorrect under the definition of parasite manipulation as a purposefully induced behavioral alteration by the parasite. The *Euderus set* lays its egg inside the gall formed around the *Bassettia pallida* larvae (Weinersmith et al., 2017). Under normal unparasitized conditions the *B.pallida*, when mature, chews out of the gall to emerge, however when parasitized by *E.set*, the *B.pallida* starts chewing an exit before it fully matures and eventually dies blocking the exit of the gall with its head (Weinersmith et al., 2017). The larvae of *E.set* continue developing until adulthood and then exit through the head of the deceased *B.pallida* (Weinersmith et al., 2017). As of now the exact mechanisms of behavioral changes in the *Bassettia pallida* are unknown and it is therefore not possible to tell if *Euderus set* uses parasite manipulation to increase its fitness.

Case studies

In most cases the mechanisms by which parasitoids wasps are manipulating host behavior are not neatly structured as in this report. Many studies have shown that the ways that parasitoid wasps manipulate host behavior is extremely complex and most of the time uses multiple systems and pathways (Libersat et al., 2018; Weinersmith, 2019). Here two case studies are presented on the parasite manipulation on a cockroach and parasitic wasp species by parasitoid wasps.

Ampulex compressa

The *Ampulex compressa*, Emerald Jewel wasp, is a solitary idiobiont parasitoid wasp that parasitizes on the American cockroach, *Periplaneta americana*. The Emerald Jewel wasp exhibits both ecto- as endoparasitic behavior as the larvae first live on the surface of the host and later enter the host's body (Weiss et al., 2014). Unlike many parasitoid wasps the *Ampulex compressa* does not directly use its venom to paralyze their host for consumption of its offspring (Arvidson et al., 2018). Instead, the wasp uses its venom, which comprises of 264 different proteins, to take control over the hosts motorial systems (Arvidson et al., 2019). First venom is injected into the head ganglia of the cockroach temporarily paralyzing the host, while the host is paralyzed the wasp performs a second precisely placed sting in the central complex, a system responsible for regulating motor behavior of the cockroach (Libersat & Gal, 2014). The stung host then proceeds by performing intense grooming behavior for around 20-25 minutes, followed by a hypokinetic state where the cockroach no longer actively moves and can be handled by the wasp (Libersat & Gal, 2014). The wasp will then bring the cockroach into its burrow where it lays a single egg on the femur of the cockroach (Weiss et al., 2014). During the development the cockroach stays in its hypokinetic state (Piek et al., 1989) and will eventually die as a result of the parasitism. The hypokinetic state, or hypokinesia is a venom induced behavioral state caused by an increased threshold for movement by the organism. The venom of the wasp does not contain any necrotic or lethal factors to ensure that the host stays in good condition during the incubation (Arvidson et al., 2019). The grooming is likely directly caused by dopamine and GABA that is present in the venom (Arvidson et al., 2019). It is currently unknown if there is an effect of the grooming behavior on the survival of the offspring, however it is suggested that grooming results in a cleaner environment for the wasp larvae.

Cotesia congregata

The gregarious parasitoid wasp *Cotesia congregata* parasitizes on multiple species of sphinx moths (Bredlau et al., 2019). Studies have been performed on the parasite manipulation of the *Cotesia congregata* on *Manducta sexta* caterpillars. The parasitoid wasp makes use of multiple mechanism to manipulate their hosts. Research has shown that the wasp injects both venom and polydnaviruses into the body of their host when injecting 50 to 150 eggs and the larvae of the wasp excrete compounds into the hosts body (Adamo et al., 2016). The combination of the venom and polydnaviruses initially repress the immune system of the caterpillar by affecting gene expression of genes related to the immune function (Amaya et al., 2005). The reduced immune function prevents the eggs and larvae of being destroyed by the host. The larvae of the wasp, being endoparasitic koinobionts, develop inside the host while the host continues behaving normally. Around 24 hours before the wasp larvae emerge from the caterpillar, the host caterpillar will stop eating and moving because of immune stimulation by cytokines that are released around the process of emerging (Adamo et al., 2016). The study suggests that the larvae secrete substances that induces this behavior (Adamo et al., 2016). The defensive strike reflex of the caterpillar remains intact, however during emergence the larvae are not targeted by this reflex. The area of emergence is temporarily desensitized, likely by the larvae, to prevent them from getting killed (Adamo et al., 2016). The larvae then spin a cocoon and continue developing under the caterpillar while the caterpillar guards the cocoon with its defensive strike reflex where it will eventually succumb to starvation (Adamo et al., 2016).

These examples show the intricate nature and complexity of parasite manipulation. The manipulation by the Emerald Jewel wasp is caused by venom injection but the venom itself contains an enormous scala of proteins that can all affect the host in different ways. The example of the *Cotesia congregata* shows that mechanisms by which parasitoid wasps manipulate their host may overlap and can be difficult to distinguish.

Discussion

Host-parasitoid interactions are a complex part of symbiotic relations that in many cases has evolved over millions of years. The goal of this study was to identify the proximate mechanisms by which parasitoid wasps manipulate the behavior of their hosts. Based on the currently available research on parasitoid manipulation, an overview was created on the complex functioning of this parasitoid strategy. Both the pathways and direct mechanisms that are used by the parasitoid wasps were categorized and other suggested behavioral manipulation mechanisms were critically reviewed on their manipulative nature. The complexity of parasitoid manipulation was presented with two case studies on host-parasitoid interactions of two different parasitoid wasp species.

Parasitoid manipulation works on endocrine and/or neural systems in the host's body. Both systems are responsible for all major internal communication and functioning of the organism (Harris-Warrick & Marder, 1991; Nelson, 2010). The systems function by distributing chemical compounds as signaling pathways, introduction of chemical compounds or the alteration of concentrations by the parasitoids can disturb and change physiological and behavioral functioning of the host. A common occurrence in parasite manipulation is the targeting of the hosts immune system pathways (Amaya et al., 2005; Shi et al., 2022). The immune system is tightly linked with both physiological and behavioral mechanisms (de Roode & Lefèvre, 2012). Parasitoid manipulation of behavioral immune responses can result in direct bodyguard manipulation which is highly beneficial for the offspring of the parasitoid (Harvey et al., 2013).

Mechanisms by which the parasitoid manipulation can be either directly caused by the parasitoid wasp, but also by symbionts that reproduce within the ovaries of the wasps. Direct manipulation by the wasp can be achieved by injecting the host with venom that interrupt the hosts natural pathways and induce behavioral responses (Arvidson et al., 2019). The venom of these wasps has different effects on the host depending on the parasitic strategy the wasp employs. Ectoparasites often induce lethargy or paralysis in their host and can affect the host immune system or development (Moreau & Asgari, 2015). Usually, the venom of endoparasites is not focused on inducing lethargy or paralysis, but more often on affecting the host immune system, development and metabolism (Moreau & Asgari, 2015). Another way of direct manipulation by the wasp is the secretion of chemical compounds by the larvae of the parasitoids (Shi et al., 2022). This strategy is most applied by endoparasitic koinobionts, the benefits of manipulation by the secretion of compounds by the larvae is that the manipulated pathways can be more consistently targeted, and that the manipulation can also be changed during the development.

Symbiont mediated parasitoid manipulation has so far been observed in the Ichneumonidae and Braconidae family. Studies discovered both RNA viruses and Polydnaviruses to be associated with manipulation of the host (Dheilly et al., 2015; Herniou et al., 2013). The mutualistic viruses replicate in the ovaries of parasitoid wasps to ensure transmission to the offspring and possibly the host. The symbiosis of Polydnaviruses and parasitoid wasps has evolved over 100 million years (Herniou et al., 2013). The viruses often target the immune system of the host by affecting transcription and gene regulation. RNA viruses have been associated with direct bodyguard manipulation in multiple studies (Dheilly et al., 2015; Mohan & Sinu, 2022).

It is important to note that not all behavioral responses that are caused by parasitoid infection and increases the parasitoids fitness are considered parasite manipulation. Examples of this are behavioral responses because of energy drain or physical agitation caused by the larvae of the wasp. Both examples trigger a response that is innate to the host and is not purposefully caused by the parasitoid.

The various pathways and mechanisms of parasitoid manipulation and the case studies of the *Cotesia congregata* and *Ampulex compressa* proof the complexity of this phenomenon. Each separate strategy can be applied by the parasitoid wasps to manipulate their host and in many cases, it is the combination of multiple approaches that lead to behavioral manipulation.

Research on the proximate mechanisms of parasitoid behavioral manipulation by wasps is very limited. There have been many studies on parasitoid manipulation however most studies are unable to identify the exact mechanisms that are applied by parasitoid wasps, this partially due to the complexity of the mechanisms. This report has summarized current findings on parasitoid behavioral manipulation by wasps, meaning that depending on future research there is likely much more to add on the exact proximate mechanisms and it is also possible that the current day categories may not encompass the findings that are yet to come.

In conclusion, this review on the proximate mechanisms of parasitoid behavioral manipulation by wasps not only summarizes strategies that are employed by parasitoid wasps, but also addresses the shortcomings in our current knowledge and parts of the co-evolutionary arms race between parasitoids, symbionts and their hosts. Understanding the proximate mechanisms of parasitoid manipulation is crucial for broadening the knowledge on evolution and the ecology of these symbiotic relationships. The knowledge on these interactions is also commercially and practically significant like in pest management as parasitoid wasps are often deployed as biological control agents. This review can act as a fundamental piece for future research, which should be focused on exploring the mechanisms of these interactions.

Afterword

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