THIAGO GECHEL KLOSS

MANIPULAÇÃO COMPORTAMENTAL DE ARANHAS POR VESPAS PARASITOIDES: HISTÓRIA NATURAL E MECANISMO DE AÇÃO

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-graduação em Entomologia, para obtenção do título de Doctor Scientiae.

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RESUMO

KLOSS, Thiago Gechel, D.Sc., Universidade Federal de Viçosa, dezembro de 2015. **Manipulação comportamental de aranhas por vespas parasitoides: história natural e mecanismo de ação**. Orientador: Carlos Frankl Sperber. Coorientador: Marcelo de Oliveira Gonzaga.

Relações entre parasitas ou parasitoides e seus hospedeiros são comuns no reino animal e representam sistemas interessantes para o entendimento de mecanismos de evolução. Um dos tópicos centrais na ecologia de organismos parasíticos é a forma com que eles ou os seus propágulos são transmitidos de um hospedeiro para outro. O sucesso na transmissão entre hospedeiros geralmente está associado a adaptações muito específicas que aumentam a probabilidade de transmissão. Uma das estratégias de transmissão parasitária mais interessante é a manipulação comportamental, onde o parasita ou parasitoide aumenta a probabilidade de transmissão por meio da alteração do comportamento do hospedeiro. Um dos exemplos de manipulação comportamental mais estudado é o das vespas da tribo Ephialtini (Insecta: Hymenoptera), que manipulam o comportamento de aranhas. As vespas injetam substâncias que paralisam temporariamente as aranhas e então depositam um único ovo nos abdomens ou cefalotórax desses hospedeiros. Após um curto período de tempo, as larvas dos parasitoides eclodem e se alimentam por meio da sucção da hemolinfa das aranhas. No estágio final de desenvolvimento larval dos parasitoides, as aranhas modificam a arquitetura das teias. Em seguida as larvas matam as aranhas e empupam no centro das teias modificadas. Informações importantes para compreender a origem e a evolução dessas interações são escassas. Na presente tese eu apresento informações sobre aspectos da história natural das espécies, investigações sobre o valor adaptativo das modificações comportamentais para a sobrevivência dos parasitoides e o mecanismo utilizado pelas vespas parasitoides

para induzir as modificações comportamentais. Utilizei como modelo de estudo as espécies Cyclosa fililineata e Cyclosa morretes (Araneae: Araneidae) que são parasitadas respectivamente por Polysphincta sp. nr. purcelli e Polysphincta janzeni (Hymenoptera: Ichneumonidae). No primeiro capítulo, descrevo o comportamento de ataque e o ciclo de vida de Polysphincta sp. nr. purcelli e Polysphincta janzeni. Sugiro que esse comportamento está relacionado com características biológicas dos hospedeiros, como observado para outras espécies. No segundo capítulo, constatei, por meio de experimento manipulativo, que as teias modificadas de C. fililineata e C. morretes aumentam a sobrevivência dos parasitoides, o que demonstra o valor adaptativo dessas teias para vespas Ephialtini. Além disso, apresento no segundo capítulo, os resultados de outro experimento manipulativo, avaliando se as modificações na estrutura das teias são causadas pela redução da condição nutricional das aranhas (i.e., provocada pela alimentação das larvas dos parasitoides). Eu demonstrei que a modificação comportamental das aranhas parasitadas não é resultante de restrição alimentar, o que interpretei como evidência adicional para a existência de um mecanismo químico responsável por induzir tais modificações comportamentais. No terceiro capítulo eu investiguei esse mecanismo químico e constatei a presença de altos níveis de ecdisona em aranhas parasitadas com comportamento alterado, em comparação com aranhas parasitadas que ainda não tinham alterado o comportamento de construção das teias e aranhas não parasitadas. Dessa forma, a modificação comportamental das aranhas possivelmente ocorre por meio da ativação do mecanismo responsável por controlar sua ecdise, o que resulta na construção de teias modificadas, que por sua vez aumentam a sobrevivência dos parasitoides.

ABSTRACT

KLOSS, Thiago Gechel, D.Sc., Universidade Federal de Viçosa, December 2015. **Behavioral manipulation of spiders by parasitoid wasps: natural history and mechanism of action.** Advisor: Carlos Frankl Sperber. Co-advisor: Marcelo de Oliveira Gonzaga.

Host-parasite and host-parasitoid relationships are common in nature and present interesting systems for gaining understanding of evolutionary mechanisms. One of the central topics in the ecology of parasitic organisms is transmission between hosts. Parasites often have specific adaptations that increase the likelihood of transmission. One of the most interesting strategies is behavioral manipulation, in which the parasite or parasitoid increases the probability of transmission by changing host behavior. One well-studied example of behavioral manipulation is that of parasitoid wasps in the Polysphinctine clade (Insecta: Hymenoptera), which manipulate spider behavior. The wasp stings a spider causing temporary paralysis, then deposit a single egg on abdomen. After a short period of time the egg then hatches into a larva that feeds on spider hemolymph. During the final stage of parasitoid larval development, the spiders modify web architecture, then the larva kills the spider and pupates in the center of the modified web. The origin and evolution of these interactions are not well understood. This thesis work investigates the natural history of two parasitoid wasp and two spider species, focusing on the adaptive value of behavioral modifications in terms of parasitoid survival and the mechanisms used by parasitoid wasps to induce spider behavioral changes. I used as model systems Cyclosa fililineata and Cyclosa morretes (Araneae: Araneidae), which are parasitized by Polysphincta sp. nr. purcelli and Polysphincta janzeni (Hymenoptera: Ichneumonidae), respectively. In the first chapter, I describe the attack behavior and life cycle of Polysphincta sp. nr. purcelli and Polysphincta janzeni. I assert that this

behavior is facilitated by biological characteristics of the host, as has been observed for other species. In the second chapter, I use a manipulative experiment to show that web modification by C. fililineata and C. morretes enhances parasitoid survival, indicating adaptive value of this behavior for Polysphinctine wasps. I also present results from a second manipulative experiment evaluating whether changes in web architecture are caused by reduced nutritional status of spiders (i.e., due to parasite exploitation of host resources). I demonstrated that behavioral modification of parasitized spiders is not the result of food restriction, which I interpret as evidence of a chemical mechanism for spider behavioral changes. In the third chapter, I investigated possible chemical mechanisms and found high levels of ecdysone in parasitized spiders with altered behavior, but not in either parasitized spiders with unaltered behavior or in nonparasitized spiders. Thus, behavioral modification in spiders may occur through activation of mechanisms controlling ecdysis, resulting in construction of modified webs and increased parasitoid survival.

1 Introdução Geral

Interações de parasitismo são frequentes entre animais de diferentes grupos taxonômicos. Um dos principais problemas enfrentados por organismos parasíticos é a transmissão dos seus propágulos de um hospedeiro para outro (MOORE, 2002; POULIN, 2010). O sucesso na transmissão entre hospedeiros geralmente está associado a estratégias que envolvem adaptações muito específicas que aumentam a probabilidade de transmissão (POULIN, 2010).

Uma das estratégias mais intrigantes de transmissão parasitária entre hospedeiros é a manipulação comportamental. Nessas estratégias, o parasita ou parasitoide aumenta a probabilidade de transmissão e/ou sobrevivência por meio da alteração do comportamento do hospedeiro (THOMAS et al. 2005). Diversos exemplos dessas modificações comportamentais são conhecidos, como: (i) grilos infectados pelo nematomorfo Paragordius tricuspidatus (Dufour, 1828) que pulam na água, local onde os parasitas adultos deixam o corpo dos grilos (THOMAS et al., 2002); (ii) parasitas trematódeos Dicrocoelium dendriticum Rudolphi (1819) que induzem formigas, que são as hospedeiras intermediárias, a subirem e fixarem-se com as mandíbulas no ápice de folhas de gramíneas, onde elas podem ser acidentalmente consumidas por mamíferos, que são os hospedeiros definitivos do parasita (CARNEY, 1969); (iii) fungos parasitas do gênero Ophiocordyceps que induzem formigas infectadas a descerem de ninhos que são localizados no dossel da floresta para a vegetação do sub-bosque, local no qual elas morrem e onde as condições para o desenvolvimento dos fungos são mais adequadas (HUGHES et al. 2011).

A maioria dos casos conhecidos de manipulação comportamental são relacionados ao aumento da aptidão dos organismos parasíticos, sendo classificados em quatro categorias: (i) “transmissão trófica”, em que as modificações comportamentais dos hospedeiros aumentam a chance de predação do indivíduo parasitado por outras espécies envolvidas no ciclo de vida do organismo parasítico; (ii) “deslocamento espacial”, envolvendo organismos parasíticos que devem sair dos hospedeiros ou liberar seus propágulos em um habitat distinto daquele normalmente ocupado pelo indivíduo parasitado; (iii) "manipulação de vetores", no qual o parasita aumenta a frequência de eventos de alimentação por seus vetores e, consequentemente, a sua transmissão a outros hospedeiros; e (iv) "manipulação envolvendo proteção", em que o comportamento alterado do hospedeiro envolve a produção de estruturas físicas, comportamentos agressivos ou a procura de micro-habitats específicos que conferem proteção ao organismo parasítico (POULIN, 2010).

Um dos exemplos mais conhecidos de modificações comportamentais que envolvem proteção do parasita/parasitoide são as interações entre aranhas e vespas parasitoides da subfamília Pimplinae (Hymenoptera, Ichneumonidae). Essa subfamília possui a tribo Ephialtini, com aproximadamente 228 espécies, que é exclusivamente ectoparasitoide de aranhas (GAULD; DUBOIS, 2006; YU et al. 2012). A indução da modificação comportamental em aranhas é conhecida para 52 espécies de vespas da tribo Ephialtini, que parasitam aranhas das famílias Nephilidae, Tetragnathidae, Araneidae, Linyphiidae, Agelenidae, Theridiidae e Dictynidae. De forma geral, as vespas fêmeas adultas dessa tribo paralisam as aranhas hospedeiras por meio da inoculação de substâncias químicas. Em seguida, elas depositam um ovo no abdômen das aranhas, que dão origem a uma larva que se

alimenta da hemolinfa das aranhas. No estágio final de desenvolvimento larval, as aranhas modificam a arquitetura das teias. Após a modificação das teias, as larvas dos parasitoides sugam completamente as aranhas e empupam no centro das teias modificadas.

Embora existam diversos registros de alterações em teias de aranhas parasitadas em várias regiões do mundo (EBERHARD, 2000a; EBERHARD, 2000b; EBERHARD, 2010; GONZAGA; SOBCZAK, 2007; GONZAGA et al., 2010; GONZAGA et al., 2015; KORENKO; PEKÁR, 2011; KORENKO et al., 2015; MATSUMOTO; KONISHI, 2007; MATSUMOTO, 2009), há carência de várias informações importantes para compreender como tais estratégias de manipulação foram moldadas ao longo da evolução das vespas Ephialtini. Por exemplo, um dos aspectos básicos para compreender o que pode determinar a associação entre uma determinada espécie de vespa e sua aranha hospedeira é verificar a relação entre o comportamento de ataque das vespas e as características biológicas dos hospedeiros. O registro desses comportamentos de ataque é conhecido apenas para sete espécies de vespas, pertencentes a três gêneros: Hymenoepimecis bicolor (Brulle 1846); Hymenoepimecis robertsei Gauld 1991; Hymenoepimecis argyraphaga Gauld 2000; Hymenoepimecis veranii Loffredo, Penteado-Dias 2009; Brachyzapus nikkoensis (Uchida 1928); Zatypota petronae Gauld 1991 e Zatypota albicoxa Walker 1874. Nestes exemplos há grandes variações nos comportamentos de ataque, e cada espécie de parasitoide apresenta características únicas, relacionadas com características comportamentais de seus hospedeiros. Entretanto, o conhecimento acumulado sobre esses comportamentos ainda é escasso considerando-se a diversidade de espécie da tribo Ephialtini, o que não permite inferir os fatores que podem determinar a associação entre os parceiros dessas interações.

Outro aspecto interessante da manipulação comportamental de aranhas é que embora diversos autores sugiram que as teias modificadas são adaptativas para as vespas, aumentando a sobrevivência das pupas dos parasitoides (NIELSEN, 1923; EBERHARD, 2000a; EBERHARD, 2001; MATSUMOTO; KONISHI, 2007; GONZAGA; SOBCZAK, 2007; WENG; BARRANTES, 2007; EBERHARD, 2010; GONZAGA et al., 2010; KORENKO; PEKÁR, 2011; KORENKO et al., 2014), essa hipótese só foi avaliada para as espécies de aranhas Agelana limbata Thorell, 1897 (Agelenidae) e Nephila clavipes (Linnaeus, 1767) (Nephilidae) (MATSUMOTO, 2009; SOBCZAK, 2013). Essa limitação de estudos não permite compreender o caráter adaptativo das modificações comportamentais em diferentes arquiteturas de teias, o que impede a afirmação de que as modificações comportamentais das aranhas são resultantes de evolução adaptativa.

O mecanismo responsável pela indução das modificações comportamentais em aranhas permanece desconhecido, o que também dificulta a compreensão da origem e evolução dos comportamentos de manipulação comportamental. Entretanto, investigações recentes indicaram que as modificações comportamentais não são consequência do estado debilitado das aranhas, após diversos dias sendo consumidas pelas larvas dos parasitoides (GONZAGA et al. 2010), mas resultado da ação direta da larva dos parasitoides (EBERHARD 2001, 2010; SOBCZAK, 2013). Especificamente com relação ao efeito direto da larva sobre as alterações comportamentais nas aranhas, investigações recentes mostraram que existem semelhanças entre teias de aranhas parasitadas e teias de aranhas não parasitadas em ecdise (ZSCHOKKE; BOLZERN, 2007; EBERHARD, 2010; GONZAGA et al. 2010; TAKASUKA et al. 2015). Essas semelhanças sugerem que o aumento do nível

do hormônio ecdisona (20-OH), provavelmente induzido pela larva dos parasitoides, resulta nas modificações das teias.

Assim, utilizando como modelo de estudo as espécies de aranhas Cyclosa fililineata (Hingston, 1932) e Cyclosa morretes (Levi, 1999), que são parasitadas respectivamente por Polysphincta sp. nr. purcelli e Polysphincta janzeni (Gauld, 1991), apresento informações sobre aspectos da história natural das espécies, o valor dessas modificações para a sobrevivência dos parasitoides e investigações sobre o mecanismo envolvido na alteração comportamental das aranhas. No primeiro capítulo apresento novas descrições sobre o comportamento de ataque de Polysphincta janzeni e Polysphincta sp. nr. purcelli, parasitando respectivamente Cyclosa morretes e Cyclosa fililineata. Além disso, descrevo o ciclo de vida dos parasitoides e a porcentagem de parasitismo nas populações de C. morretes e C. fililineata. No segundo capítulo, avalio se as modificações na teia do hospedeiro são consequência de restrição nutricional imposta pela larva do parasitoide à aranha hospedeira, e se a modificação da teia aumenta a sobrevivência do parasitoide. No terceiro capítulo da tese, apresento a primeira descrição do mecanismo químico de manipulação comportamental de aranhas, onde avalio se o hormônio ecdisona está envolvido na indução da modificação comportamental em aranhas.

Referências

CARNEY, W. P. Behavioral and morphological changes in carpenter ants harboring Dicrocoelium metacercariae. American Midland Naturalist, v.82, p.605-611, 1969.

EBERHARD, W. G. Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. Animal Behaviour, v.79, p.375-383, 2010.

EBERHARD, W. G. Spider manipulation by a wasp larva. Nature, v.406, p.255-256, 2000a.

EBERHARD, W. G. The natural history and behavior of Hymenoepimecis argyraphaga (Hymenoptera: Ichneumonidae) a parasitoid of Plesiometa argyra (Araneae, Tetragnathidae). Journal of Hymenoptera Research, v.9, p.220-240, 2000b.

EBERHARD, W. G. Under the influence: webs and building behavior of Plesiometa argyra (Araneae: Tetragnathidae) when parasitized by Hymenoepimecis argyraphaga (Hymenoptera: Ichneumonidae). Journal of Arachnology, v.29, p.354-366, 2001.

GAULD, I. D.; DUBOIS, J. Phylogeny of the Polysphincta group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider ectoparasitoids. Systematic Entomology, v.31, p.529-564, 2006.

GONZAGA, M. O.; MOURA, R. R.; PÊGO, P. T.; LEE BANG, D.; MEIRA, F. A. Changes to web architecture of Leucauge volupis (Araneae: Tetragnathidae) induced by the parasitoid Hymenoepimecis jordanensis (Hymenoptera: Ichneumonidae). Behaviour. v.152, p.181-193, 2015.

GONZAGA, M. O.; SOBCZAK, J. F. Parasitoid-induced mortality of Araneus omnicolor (Araneae, Araneidae) by Hymenoepimecis sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. Naturwissenschaften, v.94, p.223–227, 2007.

GONZAGA, M. O.; SOBCZAK, J. F.; PENTEADO-DIAS, A. M.; EBERHARD, W. G. Modification of Nephila clavipes (Araneae: Nephilidae) webs by the parasitoids Hymenoepimecis bicolor and H. robertsae (Hymenoptera: Ichneumonidae). Ethology Ecology and Evolution, v.22, p.151-165, 2010.

HUGHES, D. P.; ANDERSEN, S.; HYWEL-JONES, N. L.; HIMAMAN, W.; BILLEN, J.; BOOMSMA, J. J. Behavioral mechanisms and morphological symptoms of zombie ants dying from fungal infection. BMC Ecology. 2011. Disponível em: <http:// doi:10.1186/1472-6785-11-13>, Acesso em: 8 abril 2015.

KORENKO, S.; ISAIA, M.; SATRAPOVÁ, J.; PEKÁR, S. Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecological Entomology, v.39, p.30–38. 2014.

KORENKO, S.; PEKÁR, S. A parasitoid wasp induces overwintering behaviour in its spider host. Plos One. Retrieved from http:// doi:10.1371/journal.pone.0024628. 2011.

KORENKO, S.; SATRAPOVÁ, J.; ZWAKHALS, K. Manipulation of araneid spider web architecture by the polysphinctine parasitoid Zatypota picticollis (Hymenoptera: Ichneumonidae: Pimplinae). Entomological Science. Disponível em: http://doi:10.1111/ens.12132. 2015, Acesso em: 01 fevereiro 2015.

MATSUMOTO, R. “Veils” against predators: modified web structure of a host spider induced by an ichneumonid parasitoid, Brachyzapus nikkoensis (Uchida) (Hymenoptera). Journal of Insect Behavior, v.22, p.39-48, 2009.

MATSUMOTO, R.; KONISHI, K. Life histories of two ichneumonid parasitoids of Cyclosa octotuberculata (Araneae): Reclinervellus tuberculatus (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomological Science, v10, p.267-278, 2007.

MOORE, J. Parasites and the behaviour of animals. In: MAY, R; HARVEY, P.; GODFRAY, C.; DUNNE, J. (Eds.). Oxford Series in Ecology and Evolution. New York, U.S.A.: Oxford University Press, 2002, 1-338.

NIELSEN, E. Contributions to the life history of the Pimpline spider parasites (Polysphincta, Zaglyptus, Tromatobia) (Hym. Ichneum.). Entomologiske Meddelelser, v.14, p.137-205, 1923.

POULIN, R. Parasite manipulation of host behavior: an update and frequently asked questions. In: MITANI, J.; BROCKMANN, H. J.; ROPER, T.; NAGUIB, M.; WYNNE-EDWARDS, K. (Ed.). Advances in the study of behavior. Burlington, Canada: Academic Press, 2010, 181-186.

SOBCZAK, J. F. Estudos biológicos e ecológicos da interação entre Nephila clavipes (Araneae, Nephilidae) e o parasitoide Hymenoepimecis bicolor (Hymenoptera, Ichneumonidae, Pimplinae. 2013, 125f. Tese. (Doutorado em Ecologia e Recursos Naturais) – Pós-graduação em Ecologia e Recursos Naturais, Universidade Federal de São Carlos, São Carlos, SP, 2013.

TAKASUKA, K.; YASUI, T.; ISHIGAMI, T.; NAKATA, K.; MATSUMOTO, R.; IKEDA, K.; MAETO, K. Host manipulation by an ichneumonid spider ectoparasitoid that takes advantage of preprogrammed web-building behaviour for its cocoon protection. Journal of Experimental Biology, v.218, p.2326-2332, 2015.

THOMAS F.; SCHMIDT-RHAESA, A.; MARTIN, G.; MANU, C.; DURAND, P.; RENAUD, F. Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? Journal of Evolutionary Biology, v.15, p.356-361, 2002.

THOMAS, F.; ADAMO, S.; MOORE, J. Parasitic manipulation: where are we and where should we go? Behavioural Processes, v.68, p.185-199, 2005.

WENG, J. L.; BARRANTES, G. Natural history and larval behavior of the parasitoid Zatypota petronae (Hymenoptera: Ichneumonidae). Journal of Hymenoptera Research, v.16, p.326-335, 2007.

YU, D. S., HORSTMANN, K.; VAN ACHTERBERG, K. Taxapad. 2012. Disponível em: <http://www.taxapad.com/contact.php>, Acesso em: 8 abril 2015.

ZSCHOKKE, S.; BOLZERN, A. Erste Nachweise sowie Kenntnisse zur Biologie von Cyclosa oculata (Araneae: Araneidae) in der Schweiz. Arachnologische Mitteilungen, v.33, p.11-17, 2007.

2 Capítulo um

2.1 Attack behavior of two wasp species of the Polysphincta genus group (Hymenoptera, Ichneumonidae) on their orb-weaver spider hosts (Araneae, Araneidae)

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Attack behavior of two wasp species of the Polysphincta genus group (Hymenoptera, Ichneumonidae) on their orb-weaver spider hosts (Araneae, Araneidae)

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Abstract

Species included in the Polysphincta genus-group as far as is known, are exclusive koinobiont ectoparasitoids of spiders. These wasps attack their hosts, inflicting a temporary paralysis, and then lay one egg on the host's abdomen or prosoma. Parasitoid attack behavior is highly variable among species, including cases in which the wasp darts directly and holds the spider, as well as cases involving complex behavioral sequences. Here, we describe the attack behavior of Polysphincta sp. nr. purcelli and Polysphincta janzeni on, respectively, Cyclosa fililineata and Cyclosa morretes. All attacks occurred at night. Initially, the female wasp landing on the web hub, at the position occupied by the spider, and the spiders always escaped from this initial attack, jumping off the web or moved quickly towards the web edge Subsequently, the wasp waited for up to 14 hours at the web hub for the spider's return. The wasp then inserted its ovipositor into the mouth of the spider, after which the spider became paralyzed. The spider remained motionless for at least 30 minutes after being paralyzed. The wasp then laid one egg on the surface of host's abdomen and remained on the web for at least one hour thereafter. The lie-in-wait and attack only after the return of the host to the web hub, as well as the permanence of the wasp on the web after the attack are not frequent behaviors described for polysphinctines. Behavioral idiosyncrasies, such as those observed here, are common among polysphinctines, suggesting a high level of specific adaptive matching of polysphinctine parasitoids to their hosts' biological characteristics.

Keywords: incidence of parasitoids; orb-webs; oviposition; parasitoids.

Introduction

Ichneumonid wasps of the subfamily Pimplinae include a subgroup of 22 genera, the monophyletic Polysphincta genus-group (sensu Gauld and Dubois 2006, hereafter polysphinctines), which are distributed throughout the world (Gauld and Dubois 2006; Palacio et al. 2007). Polysphinctines are exclusive koinobiont ectoparasitoids of spiders (Gauld et al. 2002; Gauld and Dubois 2006) and are known to change their hosts' web building behavior, inducing the construction of modified structures that increase cocoon protection, reduce the probability of web collapse, and reduce risks associated with prey interception (Eberhard 2000a; Eberhard 2001; Gonzaga and Sobczak 2007; Matsumoto and Konishi 2007; Matsumoto 2009; Eberhard 2010; Gonzaga et al. 2010; Gonzaga and Sobczak 2011; Korenko et al. 2014).

Oviposition of polysphinctines requires contact between the parasitoid wasp and its potentially harmful predatory host. To minimize risk during this process, the wasps inject paralyzing venom into the host's body. Gonzaga and Sobczak (2007) showed that Hymenoepimecis veranii Loffredo and Penteado-Dias, females insert the tip of their ovipositor into the spider's mouth, probably reaching the subesophageal ganglion, and then inspect the abdomen of the host spider in search of a spot to lay an egg and for the presence of other wasp eggs. Hymenoepimecis veranii venom has an immediate effect, leaving the attacked spider motionless for at least 18 minutes. The critical stage of this process is between the initial approach and delivery of the paralyzing venom.

The few descriptions of polysphinctine attack behavior in the literature indicate that polysphinctines exhibit a high degree of variation and plasticity in their

attack strategies (Eberhard 2000b; Gonzaga and Sobczak 2007; Weng and Barrantes 2007; Matsumoto 2009; Takasuka et al. 2009; Takasuka and Matsumoto 2011; Sobczak 2013). For example, the wasps Hymenoepimecis bicolor (Brulle) and Hymenoepimecis argyraphaga Gauld, which attack Nephila clavipes (Linnaeus) (Nephilidae) and Leucage argyra (Walckenaer) (Tetragnathidae), respectively, tend to hover around target spiders before executing a direct attack and then dart rapidly at the spider, grasping it with its legs (Eberhard 2000b; Sobczak 2013). Alternatively, H. argyraphaga was observed hanging immobile from web radii in the free zone near the hub until the host spider approached (Eberhard 2000b). This behavior probably is one alternative to grasp and parasite the spider, after an unsuccessful initial attack. Similarly, H. veranii will stand immobile on barrier threads and wait until the target spider leaves its retreat to capture prey, at which point the wasp will dart for the spider (Gonzaga and Sobczak 2007). Similarly, Brachyzapus nikkoensis (Uchida) lands on the funnel web of its host spider Agelena limbata (Thorell) (Agelenidae), and also waits until the spider approaches to attack it (Matsumoto 2009).

The highest variation in attack behavior was reported within the genus Zatypota. Zatypota albicoxa Walker, for example, has at least four strategies for attacking its host Parasteatoda tepidariorum (Koch) (Theridiidae). In one strategy, known as ambush-style, the wasp hangs onto the web, pulling the thread from which it is hanging with its foreleg until the spider lifts up the thread with the hanging wasp. In its alternative ambush-style strategy, the wasp hangs motionlessly at the mid-height of the web until the spider approaches. In the climbing-style strategy, the wasp climbs the web until reaching the spider. And when employing a reclining-style attack, the wasp wanders around under the web and lays its dorsum on the floor,

pulling at a gumfooted thread with its fore- and midlegs, or leaning against the thread motionlessly with both pairs of legs directly touching a gumfooted thread, until the spider climbs down along the vertical thread, at which point it is attacked by the wasp (Takasuka et al. 2009; Takasuka and Matsumoto 2011). Meanwhile, Zatypota petronae Gauld invades the retreat of the host spider Theridion evexum Keyserling (Theridiidae), and attacks the spider inside its retreat; details regarding Z. petronae's immobilization and egg laying processes were not reported (Weng and Barrantes 2007).

Aside from the three above-mentioned wasp genera, there is no information about the attack behaviors of most of the remaining polysphinctine genera (Gauld et al. 2002; Gauld and Dubois 2006), including the species-rich genus Polysphincta. The Polysphincta genus included 27 species (Yu et al. 2012), but it comprehends a set of polysphinctines species that lack the defining features of the other wasp genera (Gauld & Dubois 2006). This fact resulted in the inclusion of several pimplines associated with spiders within this genus. Attaining a reliable definition for Polysphincta would require an expansion of our knowledge of tropical wasp fauna, which may result in a series of monophyletic genera (Gauld and Dubois 2006).

There is information on host identities for several Polysphincta species, for example: Polysphincta boops Tschek, which attacks Araniella cucurbitina (Clerck) (Araneidae) and A. opisthographa (Kulczyński) (Fritzen and Shaw 2014; Korenko et al. 2014) in Europe; Polysphincta gutfreundi Gauld, which attacks Allocyclosa bifurca (McCook) (Araneidae) and Cyclosa monteverdi Levi (Araneidae) in Costa Rica (Barrantes et al. 2008; Eberhard 2010; Gonzaga et al. 2010); Polysphincta janzeni Gauld, which has been observed parasitizing Cyclosa fililineata Hingston (Araneidae) and Cyclosa morretes Levi (Araneidae) in Brazil (Gonzaga 2004);

Polysphincta koebelei Howard, which attacks Araneus gemmoides Chamberlin and Ivie (Araneidae) (Bovee and Leech 2014) in Canada and Larinioides cornutus (Clerck, 1757) in United States (Howard, 1892); Polysphincta longa Kasparyan, which attacks Araneus angulatus Clerck (Araneidae) (Fritzen and Shaw 2014) in Poland; Polysphincta rufipes Gravenhorst, which attacks Larinioides cornutus (Clerck) (Araneidae) and Zygiella x-notata (Clerck) (Araneidae) in Germany (Schmitt et al. 2012); Polysphincta sp. nr. purcelli, which attacks Cryptachaea sp. (Keyserling) (Theridiidae) in the Brazilian Atlantic rainforest (T. G. Kloss, unpub. data); and Polysphincta tuberosa Gravenhorst, which has been observed parasitizing Araneus quadratus Clerck (Araneidae) (Nielsen 1923), A. ophistographa, Araneus diadematus Clerck (Araneidae) and A. cucurbitina (Korenko et al. 2014) in Europe. Some of these records include details of host behavioral changes (e.g. Gonzaga 2004; Eberhard 2010; Bovee and Leech 2014; Korenko et al. 2014), but female wasp behavior during attacks has reported only for P. rufipes (Schmitt et al. 2012).

The aim of this study was to provide information on the attack behavior of Polysphincta sp. nr. purcelli and of P. janzeni on C. fililineata and C. morretes, respectively. We also investigated the frequency of parasitized spiders in the populations over two seasons, encompassing the development of the parasitoids from the egg to the adult stage. The general characteristics of these two polysphinctines' attack behavior are discussed.

Materials and Methods

Field observations were conducted at the Biological Station of Santa Lucia (Estação Biológica de Santa Lúcia – EBSL) (19º57'56"S, 40º32'24"W) and the

Biological Reserve Augusto Ruschi (Reserva Biológica Augusto Ruschi – REBIO) (19º54'26"S, 40º33'11"W). Both sites are Atlantic rainforest reserves in the Santa Teresa municipality, in Espírito Santo state, Brazil.

We performed both diurnal and nocturnal visual searching of C. filililineata and C. morretes webs along all of the trails at EBSL and REBIO. When an adult polysphinctine was encountered on a web, we maintained uninterrupted observation from the initial attack (wasp landing on the hub web) until one hour after lie-in-wait attack (when the wasp grasped the spider with its legs after remained motionless at the web hub until the spider returned) successful, which was determinate by wasp oviposition. When there was no successful oviposition after lie-in-wait attack, we maintained observation until the wasp departed and in the next day, we returned to the web local to verify if the spiders had left of the web.

During these observations, we registered the behavior of wasps and spiders with Nikon D5100 camera with lens AF-S VR Micro-NIKKOR 105mm f/2.8G IF-ED, which allowed the observation of attack behaviors details. To evaluate the duration of the polysphinctine's life cycle and investigate the morphological changes in the parasitoid larva during development, we followed all host spiders subjected to oviposition (n = 4) and spider that were already parasitised by larvae in different stages (C. filililineata: n = 33; C. morretes: n = 42), until the polysphinctine larvae built their cocoons. We classified larval development in three stages: (1) first-stage larvae exhibited the absence of body divisions; (2) second-stage larvae showed segmented bodies; and (3) third-stage larvae developed characteristic dorsal tubercles, absent in previous stages. To study pupae development time, we marked 13 parasitized spiders of each species, including the two individuals of C. filililineata and two individuals of C. morretes where we observed oviposition. We observed host

individuals daily, to evaluate the period between molt of last stage larvae to pupae and parasitoid wasps emergence.

The frequency of parasitized spiders was estimated by inspecting all C. filililineata and C. morretes encountered along the trails during a 30-day period in the wet season of 2013 (November) and a 30-day period in the dry season of 2014 (July). These inspections were performed by nocturnal visual searching along all trails at EBSL and REBIO. Each spider was inspected only once within each month.

Voucher P. sp. nr. purcelli and P. janzeni specimens were deposited in a collection at Universidade Federal de São Carlos (curator, A. M. P. Dias), and specimens of C. morretes and C. filililineata were deposited in the arachnid collection of Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais (curator, A. J. Santos) in Minas Gerais, Brazil.

Results

We observed six parasitism attempts by P. nr. purcelli on C. filililineata and eight by P. janzeni on C. morretes, with two successful ovipositions for each species. Only one individual from each spider species had a parasitoid larva previously attached to its abdomen, but oviposition attempts on these two parasitized individuals were unsuccessful.

Polysphincta sp. nr. purcelli and P. janzeni presented similar initial and lie-in-wait attack behaviors. All of the initial attacks and lie-in-wait attack occurred at night on immature females of C. morretes and mature female of C. filililineata. We did not observed initial and lie-in-wait attack on males host spiders. We observed the beginning of initial attack in five occasions (two in C. filililineata and three in C.

morretes). For the others nine attack observations (four in C. filililineata and five in C. morretes) the wasp was already in the lie-in-wait attack, determinate by the wasp at the hub when we encountered it. The initial attack started with the female wasp landing on the web hub, near at the position occupied by the spider, never directly on the spider. In all observations of initial attack, the spider jumped off the web immediately or moved quickly towards the web edge. (Fig. 1a). After the initial attack, the wasp remained motionless at the web hub until the spider returned and touched the body of the wasp. We observed wasps waiting for the hosts to return for periods ranging from 30 minutes until one observation of 14 hours. Only one spider (C. morretes) not returned to the web hub after the wasp landed on the web, which occurred in this lie-in-wait attack attempt of 14 hours. The wasp departed of the web during the sunrise, after remain 14 hours on the web.

We observed that lie-in-wait attack started immediately after the spider touched the body of the wasp, when the wasp grasped the spider with its legs and the spider fought to escape, resulting in both animals moving on the web (away from the web hub), rolling one over the other. In ten observation of lie-in-wait attack (four C. filililineata and six C. morretes) the wasps failure in spider grasped, which results in the jumping of spider at the web to ground/vegetation and the wasp departed. However, we observed in the next morning, that all spiders that escaped to lie-in-wait attack returned to the hub web. After a struggle lasting a few seconds, the wasp remain positioned with its head facing the abdomen of the spider's abdomen and inserted its ovipositor into the spider's mouth (Fig. 1b), leading to immediate paralysis of the host. The wasp then inserted and withdrew its ovipositor into the spider's mouth repeatedly for about five minutes. Subsequently, the wasp apparently started to inspect the spider's body, repeatedly rubbing and jabbing the base of its

ovipositor all over the host's abdomen (near the local where the egg later is attached) for about four minutes. After this behavior, the wasp again proceeded to insert and withdraw its ovipositor into and out of the spider's mouth repeatedly for another three minutes. Finally, the wasp deposited a single egg on the antero-dorsal surface of the host's abdomen, and returned to the hub of the web (Fig. 1c), where it remained for at least an hour.

The spider recovered its mobility about 30 minutes after the oviposition, and immediately rub its posterior legs on their abdomen. Upon recovering, the spider went back to the web hub, where it touched the parasitoid wasp and then moved away quickly from the center. After a successful oviposition, the wasp remained static in the center of the web when touched by the spider, in contrast to its active response during the attack. The spider's movement towards and away from the center of the web was repeated several times, over at least an hour, when the observations were finalized.

Two days after oviposition, first-stage larvae emerged, without body divisions discernible to the naked eye. After a few days, we observed marked body divisions, which we considered to be indicative of the second stage (see Fig. 1d). During the first and second parasitoid larval stages, the host spiders of both species moved their hind legs towards the larva often. We never observed spider's legs touching a parasitizing larva, let alone removing it. However, on two occasions, second stage larvae, parasitizing C. morretes hosts, disappeared from one day to another.

The third stage (Fig. 1e and f), was originated two days before the wasp larvae kill the spider host. We observed that dorsal tubercles are used by larvae to hold onto the web, after killing the host spider (see arrow in Fig. 1f). On the last day

of this larval stage, the host spiders built a modified web (Kloss et al. 2016). After construction of the modified web, the parasitoid larvae attached themselves to the web hub and proceeded to consume the host spiders. (Fig. 1f). Then, the parasitoid larvae initiated spinning of a cocoon at the center of the web or into the stabilimentum (Fig. 1g). The period of larval development, from the egg stage up to cocoon building, varied from 23 to 26 days (n = 4). The adult polysphinctine emerged 11 to 14 days (12.3 ± 0.91, mean ± SE) after its cocoon had been built (Fig. 1h, P. janzeni: n = 13; Polysphincta sp. nr. purcelli: n = 13).

The frequency of parasitism differed between the seasons evaluated. In the wet season, we registered 111 C. filililineata webs and 124 C. morretes webs, of which 11 C. filililineata (9.90 %) and five C. morretes (4.03 %) were parasitized. In the dry season, we registered 102 C. filililineata individuals and 124 C. morretes individuals, of which 15 (14.70 %) C. filililineata and 29 (23.38 %) C. morretes were parasitized.

Discussion

In the present study, we observed three main aspects of P. nr. purcelli and P. janzeni oviposition behavior: (i) fly and land on the web hub, at the position occupied by the spider, and wait a long time for the spider to return; (ii) attack the host after it has returned to the hub and touched the wasp; and (iii) remain on the web for an extended period of time after attacking the spider.

The wasp behavior of waiting for the host spider's return, instead of immediately attacking it, is probably linked to the high value of the web for these host spider species. Cyclosa filililineata and C. morretes build characteristic detritus

stabilimenta on their webs. Stabilimenta are thought to reduce their conspicuity to visually oriented predators (Gonzaga and Vasconcellos-Neto 2005) and prey insects (Tan and Li 2009). The columns of detritus are built gradually, with a progressive accumulation of food debris and other particles that fall unto the web (Gonzaga and Vasconcellos-Neto 2005; Gonzaga and Vasconcellos-Neto 2012). Spiders may remain better concealed in long structures with about the same width of their bodies. Thus, abandoning one web to move to another site would require that the spiders rebuild the stabilimentum, during which time they would be exposed to predators. The parasitoid wasps may favor waiting on webs with a completed stabilimentum to exploit this risk avoidance behavior in their hosts. The presence of a detritus stabilimentum at the hub of the web, concealing spider position, may also be important to influence the indirect attack behavior by P. janzeni and Polysphincta sp. nr. purcelli. Direct attacks by polysphinctines were previously observed only against spider hosts that remain exposed and clearly visible at the hub of their orbs, such as Nephila clavipes (Sobczak 2013) and Leucauge argyra (Eberhard 2000b). In the cases in which host spiders are protected by a complex structure of silk threads (e.g. Manogea porracea - Sobczak et al. 2009) or inside shelters (e.g. Araneus omnicolor - Gonzaga and Sobczak 2007) attacks involve landing on the web or retreat to chase the host or to wait until it leaves the protected position. Polysphincta sp. nr. purcelli and P. janzeni may be unable to detect the position occupied by the spider within the detritus column accurately enough to conduct a direct attack, adopting an alternative strategy of waiting the spider at the hub after cause an initial disturbance. In fact, tests calculating color contrasts between spiders and their stabilimenta conducted using other Cyclosa species as models showed that spiders are invisible to hymenopterans at close distances (Chou et al. 2005; Tan and Li 2009).

Attacking the host spider only after the spider had returned to the hub and touched the wasp, as opposed to pursuing it aggressively straight away, may reduce the probability that the host spider can escape by jumping away. In the field, we observed frequent jumping behavior in both C. filililineata and C. morretes in response to the approach of a researcher, moths hovering over the web, and Mimetidae predator spiders climbing on the web.

The postoviposition behavior of returning to the web hub and remaining there for at least an hour, instead of leaving the web immediately, has never been registered for polysphinctines. The function of this behavior is not known, however, given that we observed the spiders attempting to remove the deposited eggs upon recovering from paralysis, by moved their hind legs towards the abdomen (which also can explain the larvae disappearance, observed in two C. morretes – see Results), the perpetuity of the wasp may be a strategy to assure a successful oviposition. However, the existence of some visual or chemical mechanism used by the wasps to detect egg removal is not known. In addition, to spider efforts to remove the egg, other species of the polysphinctine are known to practice this behavior (Eberhard 2000b; Gonzaga and Sobczak 2007; Takasuka and Matsumoto 2011). Conversely, it could be argued that the time spent by the mother wasp on the web after oviposition is likely too short to significantly decrease the probability of egg mortality. Other parasitoids have been documented removing not only recently laid eggs, but also larvae (Takasuka and Matsumoto 2011), with the risk of eviction being dispersed along the whole period of larval development.

Although some characteristics of P. nr. purcelli and P. janzeni oviposition behavior appear to be unique among polysphinctines, they exhibited some behavioral sequences that may be widespread among polysphinctines. The insertion of the

ovipositor into the mouth of the host, and consequent paralysis of the spider, are one of several ways known in H. argyraphaga (Eberhard 2000b) and H. veranii (Gonzaga and Sobczak 2007). The behavior of groping the host spider's abdomen after paralysis has also been described for other species. This behavior, which has been interpreted as being related to searching for pre-existing larvae or eggs (Takasuka and Matsumoto 2011), was observed for Hymenoepimecis (Eberhard 2000b; Gonzaga and Sobczak 2007; Fincker 1990), Reclinervellus (Matsumoto and Konishi 2007), and Zatypota (Takasuka et al. 2009). Although not proven, nocturnal attacks behaviours, such as those described here, were suggested for Hymenoepimecis and Acrotaphus (Gauld and Dubois 2006).

In conclusion, the present observations suggest that the oviposition behavior of P. nr. purcelli and P. janzeni may be related to the characteristic reactions of their hosts. The oviposition behavior sequences of other polysphinctine species also seem to be well-suited to specific behaviors or web architectures of their hosts. Hymenoepimecis veranii, for example, would probably fail to subdue its host within the host's curled leaf shelter. Thus, instead of invading the host's shelter, H. veranii waits outside of the host's retreat until the spider leaves spontaneously to capture an intercepted insect. These fine-tuned interactions probably occur in others polysphinctine species and may restrict the range of host species used by each parasitoid.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

Barrantes G, Eberhard WG, Weng JL (2008) Seasonal patterns of parasitism of the tropical spiders Theridion evexum (Araneae, Theridiidae) and Allocyclosa bifurca (Araneae, Araneidae) by the wasps Zatypota petronae and Polysphincta gutfreundi (Hymenoptera, Ichneumonidae). Rev Biol Trop 56:749-754

Bovee J, Leech R (2014) Araneus gemmoides (Araneae: Araneidae) death by a parasitoid (Hymenoptera: Ichneumonidae). Bull Entomol Soc Can 46:86-91

Chou IC, Wang PH, Shen PS, Tso IM (2005) A test of prey-attracting and predator defence functions of prey carcass decorations built by Cyclosa spiders. Anim Behav 69: 1055–1061

Eberhard WG (2000a) Spider manipulation by a wasp larva. Nature 406:255-256

Eberhard WG (2000b) The natural history and behavior of Hymenoepimecis argyraphaga (Hymenoptera: Ichneumonidae) a parasitoid of Plesiometa argyra (Araneae, Tetragnathidae). J Hym Res 9:220-240

Eberhard WG (2001) Under the influence: webs and building behavior of Plesiometa argyra (Araneae, Tetragnathidae) when parasitized by Hymenoepimecis argyraphaga (Hymenoptera, Ichneumonidae). J Arachnol 29:354-366

Eberhard WG (2010) Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. Anim Behav 79:375-383

Fincke OM, Higgins L, Rojas E (1990) Parasitism of Nephila clavipes (Araneae, Tetragnathidae) by an ichneumonid (Hymenoptera, Polysphinctini) in Panama. J Arachnol 18:321-329

Fritzen NR, Shaw MR (2014) On the spider parasitoids Polysphincta longa Kasparyan and P. boops Tschek (Hymenoptera, Ichneumonidae, Pimplinae), with the first host records of P. longa. J Hym Res 39:71-82

Gauld ID, Dubois J (2006) Phylogeny of the Polysphincta group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider ectoparasitoids. Syst Entomol 31:529-564

Gauld ID, Wahl DB, Broad GR (2002) The suprageneric groups of the Pimplinae (Hymenoptera: Ichneumonidae): a cladistic re-evaluation and evolutionary biological study. Zool J Linn Soc-Lond 136:421-485

Gonzaga MO (2004) Funções e variabilidade estrutural dos estabilimentos construídos por Cyclosa fililineata Hingston 1932 e Cyclosa morretes Levi, 1999 (Araneae: Araneidae). [Ph.D. thesis], Universidade Estadual de Campinas, Brazil

Gonzaga MO, Sobczak JF (2007) Parasitoid-induced mortality of Araneus omnicolor (Araneae, Araneidae) by Hymenoepimecis sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. Naturwissenschaften 94:223–227

Gonzaga MO, Sobczak JF (2011) Behavioral manipulation of the orb-weaver spider Argiope argentata (Araneae: Araneidae) by Acrotaphus chedelae (Hymenoptera: Ichneumonidae). Entomol Sci 14:220-223

Gonzaga MO, Sobczak JF, Penteado-Dias AM, Eberhard WG (2010) Modification of Nephila clavipes (Araneae: Nephilidae) webs by the parasitoids Hymenoepimecis bicolor and H. robertsae (Hymenoptera: Ichneumonidae). Ethol Ecol Evol 22:151-165

Gonzaga MO, Vasconcellos-Neto J (2005) Testing the functions of detritus stabilimenta in webs of Cyclosa fililineata and Cyclosa morretes (Araneae: Araneidae): Do they attract prey or reduce the risk of predation? Ethology 111:479-491

Gonzaga MO, Vasconcellos-Neto J (2012) Variation in the stabilimenta of Cyclosa fililineata Hingston, 1932, and Cyclosa morretes Levi, 1999 (Araneae: Araneidae), in Southeastern Brazil. Psyche. doi: 10.1155/2012/396594

Howard LO (1892) The Hymenopterous parasites of spiders. Proc Entomol Soc Wash 2:290-302

Kloss TG, Gonzaga MO, Roxinol JAM, Sperber, CF (2016) Host behavioural manipulation of two orb-weaver spiders by parasitoid wasps. Anim Behav 111:289-296

Korenko S, Isaia M, Satrapová J, Pekár S (2014) Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecol Entomol 39:30-38

Matsumoto R (2009) “Veils” against predators: modified web structure of a host spider induced by an ichneumonid parasitoid, Brachyzapus nikkoensis (Uchida) (Hymenoptera). J Insect Behav 22:39-48

Matsumoto R, Konishi K (2007) Life histories of two ichneumonid parasitoids of Cyclosa octotuberculata (Araneae): Reclinervellus tuberculatus (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomol Sci 10:267-278

Nielsen, E. (1923). Contributions to the life history of the Pimpline spider parasites (Polysphincta, Zaglyptus, Tromatobia) (Hym. Ichneum.). Entomol medd 14:137-205

Palacio E, Sääksjärvi IE, Vahtera V (2007) Lamnatibia, a new genus of the Polysphincta group of genera from Colombia (Hymenoptera: Ichneumonidae; Pimplinae). Zootaxa 1431:55-63

Schmitt M, Richter D, Göbel D, Zwakhals K (2012) Beobachtungen zur Parasitierung von Radnetzspinnen (Araneidae) durch Polysphincta rufipes (Hymenoptera: Ichneumonidae). Arachnol Mitt 44:1-6

Sobczak JF (2009) Modificações comportamentais em aranhas orbitelas (Araneae: Tetragnathidae, Nephilidae, Araneidae) atacadas por vespas parasitoides (Ichneumonidae, Pimplinae, Polysphinctini). [Master thesis], Universidade Federal de São Carlos, São Carlos, Brazil

Sobczak JF (2013) Estudos biológicos e ecológicos da interação entre Nephila clavipes (Araneae, Nephilidae) e o parasitoide Hymenoepimecis bicolor

(Hymenoptera, Ichneumonidae, Pimplinae). [Ph.D. thesis], Universidade Federal de São Carlos, São Carlos, Brazil

Takasuka K, Matsumoto R (2011) Liying on the dorsum, a unique host attacking behavior of Zatypota albicoxa (Hymenoptera, Ichneumonidae). J Ethol 29:203–207

Takasuka K, Matsumoto R, Ohbayashi, N (2009) Oviposition behavior of Zatypota albicoxa (Hymenoptera, Ichneumonidae), an ectoparasitoid of Achaearanea tepidariorum (Araneae, Theridiidae). Entomol Sci 12:232–237

Tan EJ, Li D (2009) Detritus decorations of an orb-weaving spider, Cyclosa mulmeinensis (Thorell): for food or camouflage? J Exp Biol 212:1832-1839

Weng JL, Barrantes G (2007) Natural history and larval behavior of the parasitoid Zatypota petronae (Hymenoptera: Ichneumonidae). J Hym Res 16:326-335

Yu DS, van Achterberb C, Horstmann K (2012) Taxapad 2012, World Ichneumonoidea 2011, Taxonomy, biology, morphology and distribution. http://www.taxapad.com. Accessed 30 April 2015

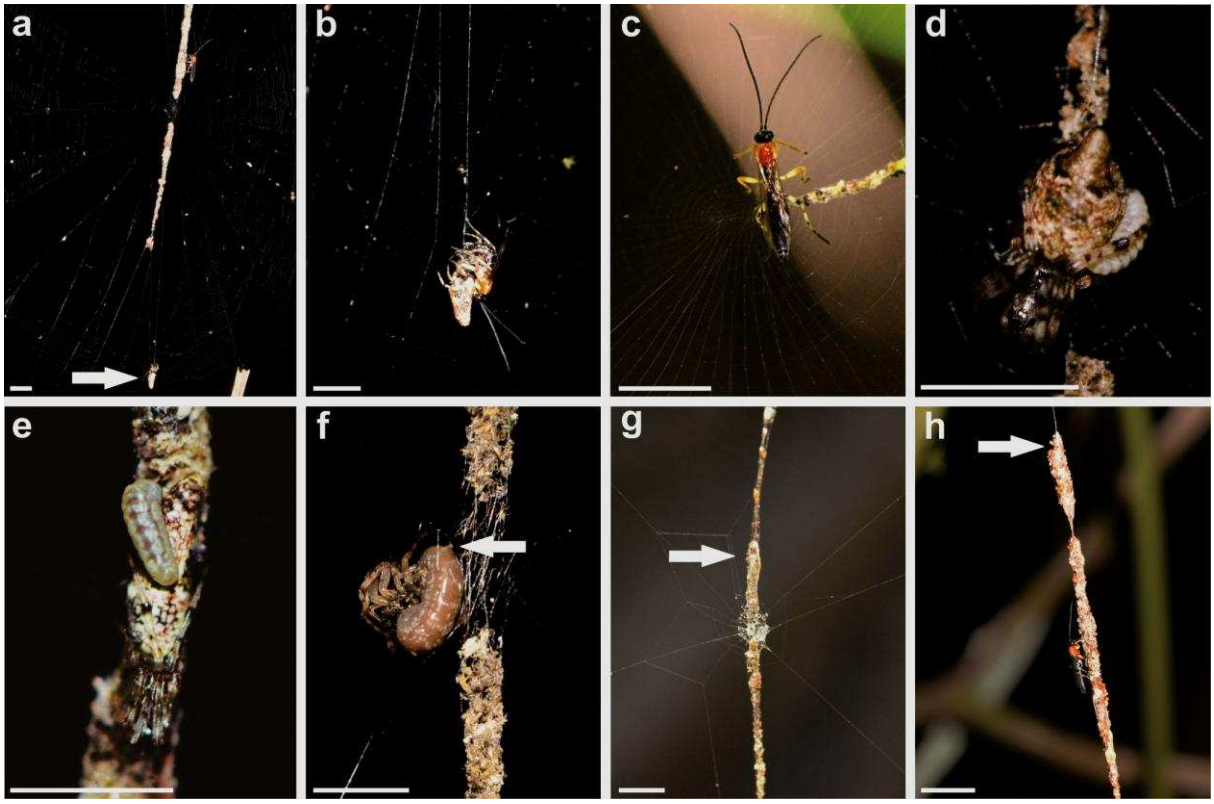

Fig. 1 Life cycle of the parasitoids P. nr. purcelli and P. janzeni: **a** Adult P. nr. purcelli landing on the center of the web and the host spider escaping (arrow) to the edge of the web; **b** Immobilization of an adult female C. fililineata host for P. nr. purcelli; **c** P. janzeni individual at the center of a C. morretes web postattack; **d** Second-stage larva of P. janzeni on its C. morretes host; **e** Third-stage larva of P. nr. purcelli on its adult female C. fililineata host; **f** Parasitoid larva of P. nr. purcelli attached to the center of the cocoon web by small hooks on its dorsal pseudopods (arrow) after killing the host spider; **g** P. janzeni cocoon (arrow) in center of the stabilimentum of a C. morretes cocoon web; **h** Adult male P. nr. purcelli emerging from a cocoon (arrow) located on a C. fililineata cocoon web. Scale bars: 0.5 cm.

3 Capítulo dois

3.1 Host behavioural manipulation of two orb-weaver spiders by parasitoid wasps

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Host behavioural manipulation of two orb-weaver spiders by parasitoid wasps

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Parasitic infection or tissue consumption by parasitoids typically leads to several phenotypic alterations in hosts, including distinct changes in behavioural, morphological, or life history traits. Some of these changes may not result solely from host responses, but actively induced changes by parasites/parasitoids to manipulate the host to acquire some benefit (e.g. parasite/parasitoid survivorship or dispersal ability). Field experiments investigating the effects of host behavioural alterations on parasite or parasitoid survivorship and the mechanisms involved in these changes are important for confirmation of behavioural manipulation. In the present study, we examined web design modification in the host spider species Cyclosa fililineata and Cyclosa morretes, which are attacked by the polysphinctine ectoparasitoide wasps Polysphincta sp. nr. purcelli and Polysphincta janzeni, respectively. We tested whether changes in orb spider web design (1) result from nutritional restrictions imposed by parasitoids and (2) increase the chances of adult wasp emergence from the cocoon. Furthermore, we describe changes in web design consistent with parasitoid larval development. Results, for both spider species, showed that web design modifications occurred only during the night preceding host spider death, and that modifications involved a reduction in the number of spirals and radii of orb webs. Food restriction did not generate web design modification, suggesting that observed changes in host behaviour resulted from direct actions of parasitoid larvae rather than as a by-product of nutritional deficiencies. Rain events were the major factor causing web rupture and subsequent parasitoid mortality. Modifications in web design reduced the frequency of web ruptures, increasing parasitoid adult emergence, and thus survivorship probability, during the pupal stage. These results confirm that web modifications by spiders are adaptive to parasitoid wasps.

Keywords: behaviour manipulation, cocoon web, Cyclosa fililineata, Cyclosa morretes, orb web, parasitoid fitness, Polysphincta

Some parasitic organisms induce changes in their host's behaviour (Thomas, Adamo, & Moore, 2005). Alterations in parasitized host phenotypes might constitute nonadaptive infectious by-products, host adaptations to reduce damages promoted by the parasite, or parasitic manipulation to facilitate transmission to other hosts (Levri, 1999; Poulin, 2010; Schmid-Hempel, 2011).

Poulin (1995) established four criteria to determine whether behavioural modifications are adaptive in parasites: (1) behavioural changes must be complex; (2) the changes must show conformity to a priori expectations based on their purported function; (3) the occurrence of convergence between unrelated parasite lineages with respect to the type of behavioural changes induced in the host; and (4) parasites or parasitoids must achieve increased transmission success or survivorship probability when exploiting the new behaviour of their hosts. However, Poulin (2010) later emphasized that these criteria (Poulin, 1995), as a whole, were overly conservative, and that the most convincing evidence in favour of adaptation would be experimental demonstration of fitness benefits to the parasite.

Several examples of adaptive manipulation in parasites have been documented. Poulin (2010) classified these examples in the following four categories: (1) ‘trophic transmission’, in which behavioural modification increases the parasitized host's predation susceptibility by another species involved in the parasite's life cycle; (2) ‘spatial displacement’, where parasites must exit the host or release their propagules in a habitat distinct from that typically occupied by the host; (3) ‘manipulation of vectors’, in which the parasite increases the frequency of its

vector's feeding events, and consequently, their transmission to other host individuals; and (4) ‘manipulation involving protection’, where the host's altered behaviour involves the production of physical structures, aggressive routines or searching for specific microhabitats conferring protection to the parasitoid. Spider host and ichneumonid wasp interactions can be included in this last category. Parasitized individuals usually build peculiar web types (cocoon webs), appropriate for parasitoid cocoon attachment but inefficient for intercepting insects (Eberhard, 2000a, 2000b; Gonzaga & Sobczak, 2007).

Alterations in web designs arising via parasite host pressure in several lineages of web-building spiders attacked by ichneumonids of the Polysphincta clade (Gauld & Dubois, 2006) have been reported (Eberhard, 2000a, 2001, 2010a; Gonzaga, Moura, Pêgo, Lee Bang, & Meira, 2015; Gonzaga & Sobczak, 2007; Gonzaga, Sobczak, Penteado-Dias, & Eberhard, 2010; Korenko & Pekár, 2011; Korenko, Satrapová, & Zwakhals, 2015; Matsumoto, 2009; Matsumoto & Konishi, 2007). The modified spider behaviour usually involves (1) the expression of repeated routines of normal web building behaviour (Eberhard, 2010b), (2) a reduction in the number of web components important to intercept insects (Gonzaga & Sobczak, 2007; Gonzaga et al., 2010) and, sometimes, (3) the construction of web parts normally produced only by juvenile individuals (Gonzaga, Moura, et al., 2015).

Although several authors have suggested that cocoon webs can enhance survival of parasitoid pupae by reducing mortality from predators and abiotic factors (Eberhard, 2000a, 2001, 2010a; Gonzaga & Sobczak, 2007; Gonzaga et al., 2010; Korenko, Isaia, Satrapová, & Pekár, 2014; Korenko & Pekár, 2011; Matsumoto & Konishi, 2007; Nielsen, 1923; Weng & Barrantes, 2007), only two experimental studies have compared mortality in modified and unmodified structures. Matsumoto

(2009) reported that Agelena limbata (Agelenidae) individuals spin a specific structure (a veil at the entrance of the funnel web) when parasitized by Brachyzapus nikkoensis (Ichneumonidae) that blocks entry by crawling predators, such as ants, and thus reduces predation risk of the parasitoid pupae. Sobczak (2013) verified that cocoons of Hymenoepimecis bicolor (Ichneumonidae), a parasitoid of Nephila clavipes (Nephilidae), were more likely to persist until the emergence of adult wasps, when attached to tridimensional cocoon webs.

The mechanisms responsible for behavioural manipulation of spiders also remain to be described. One possibility is that alterations, such as reduction of web complexity, might arise from nutritional restrictions of parasitized spiders. Spiders have the ability to invest in webs based on nutritional status (Baba & Miyashita, 2006; Mayntz, Toft, & Vollrath, 2009). It is therefore possible that debilitated individuals, after several days of being consumed by parasitoid larvae, might reduce their energy in web investment. Contrary to this prediction, Gonzaga et al. (2010) found no evidence of web design modification induced by food deprivation in N. clavipes parasitized by H. bicolor. However, this result does not provide suitable evidence to discount the relationship between nutritional deficiency and web modification in parasitized spiders.

In this study, we conducted a field investigation of web design modifications in two orb-weaver spiders, Cyclosa fililineata and Cyclosa morretes (Araneidae) parasitized by the polysphinctine wasps Polysphincta sp. nr. purcelli and Polysphincta janzeni (Ichneumonidae), respectively. Through manipulative field experiments, we evaluated the following hypotheses: (1) web modification results from host debilitation, due to nutritional deficiencies; and (2) web modification is an adaptation enhancing pupal survival. For the first hypothesis, we predicted that the

number of web spirals and radii constructed by spiders maintained under food deprivation would differ from spiders maintained under no food restrictions, constructing normal webs (i.e. similar to the number of spirals and radii of cocoon webs). For the second hypothesis, we predicted that parasitoid survival would be higher on cocoon webs, compared to unmodified webs. We also investigated web design modifications during parasitoid larvae development.

METHODS

Study Area

Field observations and experimental manipulations were conducted at the Estação Biológica de Santa Lúcia (19º57′56′′S, 40º32′24′′W) and Reserva Biológica Augusto Ruschi (19º54′26′′S, 40º33′11′′W). Both Atlantic rainforest nature reserves are located in the Santa Teresa municipality, Espírito Santo State, Brazil. These areas are classified as dense ombrophylous mountain forests (Velozo & Góes-Filho, 1982).

Study Species

The C. fililineata spider exhibits a wide geographical range from Panama to Argentina, and C. morretes is endemic to Brazil (World Spider Catalog, 2015). Both species add detritus stabilimenta at the hub of their orb webs. Gonzaga and Vasconcellos-Neto (2012) reported five stabilimentum types: (1) linear structures containing silk and debris; (2) incomplete columns characterized by one to many

blocks of silk and debris; (3) complex structures, often resembling a large spider; (4) spiral shapes composed only of silk; and (5) linear silk structures.

The parasitoid wasp P. janzeni was previously known from primary tropical rainforest near Estación Sirena in the Corcovado National Park, Osa Península, Costa Rica (Gauld, 1991) and the Atlantic rainforest in Parque Estadual de Intervales, São Paulo, Brazil, where it also attacks C. filililineata (Gonzaga, Cardoso, & Vasconcellos-Neto, 2015). The potentially closely related P. purcelli (Gauld, 1991) was reported from Belize and Costa Rica (Gauld,1991; Yu, Horstmann, & Van Achterberg, 2012).

Polysphincta sp. nr. purcelli attack only adult females of C. filililineata and P. janzeni attack only immature individuals of the larger species C. morretes. Female wasp paralyses its host by venom injection and then lays one egg on the surface of its abdomen. The ectoparasitic larvae feed for 21e24 days on the spider host by sucking the spider's haemolymph, which induces the host spider to build a cocoon web. The wasp larvae subsequently kill the host and build a cocoon that is suspended from the hub of its host's modified orb web (T. G. Kloss, personal observation).

Web Modification

We analysed web structure of mature C. filililineata and immature C. morretes female hosts to evaluate web design changes, comparing webs constructed by parasitized and nonparasitized spiders. We analysed 30 C. filililineata females parasitized by P. sp. nr. purcelli and 18 nonparasitized females. Among the parasitized C. filililineata females, nine carried first-stage parasitoid larvae, 12 carried second-stage larvae and nine carried third-stage larvae. We also analysed 42 female C. morretes parasitized by P. janzeni; six carrying first-stage larvae, 20 carrying

second-stage larvae and 16 carrying third-stage larvae. Sixteen C. morretes females were nonparasitized.

We determined the specific parasitoid larval stage to evaluate web changes throughout larval development by using web comparisons from spiders carrying larvae in different developmental stages. Larvae were classified into the following three stages: (1) first-stage larvae exhibited the absence of body divisions; (2) second-stage larvae showed segmented bodies; and (3) third-stage larvae developed characteristic dorsal tubercles, absent in previous stages.

We quantified structural web changes in the field by first covering the webs with water, then photographing them. We evaluated changes in radii and spiral number, variables previously used to describe web alterations resulting from spider host and larval parasitoid interactions (e.g. Eberhard, 2010a; Gonzaga & Sobczak, 2007; Gonzaga et al., 2010).

We investigated and statistically analysed web design modification throughout larval parasitoid development. We applied ANOVA to compare radii and spiral number among webs constructed by host spiders parasitized by third-stage larvae and webs constructed by nonparasitized spiders. We adjusted separate generalized linear models (GLM) for each response variable/spider species with Poisson distributions, corrected for overdispersion when necessary. We evaluated significance with contrast analyses, using F test for overdispersed data, following Zuur, Ieno, Walker, Saveliev, and Smith (2009).

Food Deprivation Experiment

We evaluated whether food deprivation was responsible for web

modifications by collecting 32 nonparasitized C. filililineata and 16 nonparasitized C. morretes. We transferred each individual to a wooden frame (26 x 22 cm) (see Supplementary Fig. S1), suspended by a rope 1 m above ground level. The frames were hung 40 cm apart, along four rows (spaced by 2 m). Each transferred spider remained where it was placed and initiated web line production immediately after transference. We chose the first five web lines produced by the spider and with a tweezer, attached the lines to different locations on the wooden frame. This was done to guarantee that web building was limited to the wooden frame.

When the web was completed, each frame/web was bagged in a white organza fabric bag (80 x 50 cm), inserted from above, and tied at the bottom to prevent prey entrance. Two 15 cm long transverse wood pieces were nailed to each frame corner to ensure that the bag would not disrupt the spider web. An 80 cm long Velcro strip was present on the lateral side of each bag to facilitate opening and closing during food supply manipulation and photography (see Supplementary Fig. S1). The spiders from each species were subdivided into two groups for manipulation of food availability. The first group (C. filililineata, N = 15; C. morretes, N = 8) was fed one termite (Nasutitermes obscurus) (Isoptera: Termitidae) soldier daily for 21 days. Prey length was approximately 0.4 cm, corresponding to the body length of spiders used in the experiments. The quantity of food for this first group was established based on previous studies showing that a normal developmental rate is achieved in other species of juvenile orb weaver using less food than was used in our experiment (i.e. our food level is more than sufficient to produce desired results) (Higgins & Goodnight, 2010). Furthermore, orb web spiders are usually successfully reared under laboratory conditions while being fed only once per week (Zschokke & Herberstein, 2005). The second group (C. filililineata, N = 17; C. morretes, N = 8) was

starved over the entire 21-day period. All spiders were sprayed with water daily. We established the duration of the evaluation period based on P. sp. nr. purcelli and P. janzeni larval development period, measured previously in the field during observations of the parasitoid's life cycle (T. G. Kloss, personal observation). We evaluated differences between treatments by comparing web structure on day 1 and day 21 of the experiment, using repeated measures ANOVA, considering individual spiders as random effects. We adjusted generalized linear mixed models (GLMM) with Poisson distributions and random intercepts, corrected for overdispersion when necessary. Response variables were radii and spiral number, with response variables and spider species analysed in separate models. Food deprivation was the explanatory factor, consisting of two levels (fed and unfed). Significance was evaluated using χ^2 for Poisson distributions, following Zuur et al. (2009).

Pupae Survivorship in Unmodified and Cocoon Webs

We evaluated effects of cocoon web construction on parasitoid survival by marking 28 C. fililineata and 30 C. morretes webs. All webs chosen contained spiders parasitized by first- or second-stage larvae. Webs were monitored daily until web modification was observed. After web modification, we divided the spiders into two groups. Group I consisted of parasitized spiders, which we removed from their webs and transferred to unmodified webs; the resident spider had been removed and released on vegetation 50 m away from its web. Group II spiders were removed from their original webs and placed into 2 ml plastic-capped vials for 1 min, then transferred back to the same web from which they were removed; this was to control for possible manipulation effects. We used the original cocoon webs for spider

transference, instead of a different cocoon web, because moulting of parasitoid larvae to the final stage occurs asynchronously. Therefore, other cocoon webs were generally unavailable for larvae transfer when needed. Soon after manipulation, all parasitized host spiders died and the parasitic larvae hooked firmly onto the host web and pupated. Treatment levels (i.e. cocoon web and normal web) were assigned alternately, so as to avoid confounding climatic and seasonal effects. In all cases, P. sp. nr. purcelli and P. janzeni parasitoid larvae constructed cocoons on the day that larvae were transferred to normal webs or reintroduced to modified webs. Spider activity on webs was thus minimized prior to death, reducing the risk of spider manipulation affecting web structure. We monitored webs daily until adult parasitoid emergence. We determined the following observations as failure in parasitoid development (parasitoid death): (1) the cocoon remained attached to the host web, but presented a large lateral orifice on its surface (which differed from the orifice used for adult emergence), caused by a parasitoid predator; (2) the web collapsed and the cocoon fell to forest floor or on surrounding vegetation; and (3) the cocoon disappeared from a damaged host web. We compared parasitoid survival between cocoon webs and normal webs, separately for each spider host, using survival analyses with Weibull hazard distribution (Crawley, 2013). We adjusted a full model with treatment (cocoon versus normal web), accumulated rain volume (mm), and an interaction term of rain and treatment to evaluate eventual effects of web damage caused by rain. The full model was simplified by deletion of nonsignificant effects. Accumulated rain data, collected for each web during the experiment, was obtained by pluviometers (Apager, model NR 316/80) located in each of the two study areas.

Ethical Note

Experimental manipulation of spiders and field work were performed with permission from the System of Authorization and Information on Biodiversity e SISBIO/ICMBio (Authorization No. 34711/1, Brazil) and complied with the current legal and ethical requirements for animal welfare. To minimize adverse impacts on subjects and to enhance welfare, we restricted the length of food deprivation periods to the shortest time necessary to simulate the typical length of time required for parasitoid larval development (i.e. 21 days). Food deprivation experiments were conducted at the site of spider occurrence in the field, so as to prevent injuries and maintain animal welfare by avoiding unusual changes in temperature and humidity. No spiders died during the food deprivation experiment, which was expected due to previous knowledge that spiders can tolerate long periods of food restriction (see Anderson, 1974; Vollrath & Samu, 1997). This tolerance is achieved by a reduction in metabolic rate, an adaptive trait in conditions of scarce prey (Anderson, 1974; Jensen, Mayntz, Toft, Raubenheimer, & Simpson, 2011). All food-deprived spiders were fed for 3 days after the deprivation experiment ceased, and then released in the field. Only the minimum required number of soldier termites were collected (as a food source for spiders) to prevent abusive disturbance of the termite colony.

RESULTS

Web Modification

Nonparasitized webs of both species presented orb with varying numbers of radii and

spirals (Fig. 1a, c), which were absent in cocoon webs. One cocoon web pattern was observed for C. filililineata (Fig. 1b), and two cocoon web patterns were found in C. morretes (Fig. 1d, e). The most frequent pattern for C. morretes (N = 14) showed similarities to C. filililineata in one cocoon web pattern. However, the second C. morretes pattern (N = 2) exhibited hub loops and V radii, resulting in a doubling of the line number in each radius (arrow Fig. 1e), which were not observed in C. filililineata cocoon webs or in the most common C. morretes cocoon web pattern. All cocoon webs were constructed after the larvae moulted into the third stage (Fig. 2), just before the host spider was killed and its abdomen consumed. For both host spider species, cocoon webs parasitized by third-stage larvae showed significant reductions in the number of radii (ANOVA: C. filililineata: $F_{1,46} = 94.2$, $P < 0.001$; C. morretes: $F_{1,56} = 184.8$, $P < 0.001$) and spirals (ANOVA: C. filililineata: $F_{1,46} = 127.8$, $P < 0.001$; C. morretes: F1,56 = 127.7, $P < 0.001$) relative to webs of nonparasitized spiders and webs of spiders parasitized by first- and second-stage larvae.

Food Deprivation Experiment

We did not detect significant effects of food deprivation on web structure for either host spider species. Following 21 days, webs of fed and unfed C. filililineata showed a similar number of radii (repeated measures ANOVA: $\chi^2_1 = 1.65$, $P = 0.19$; Fig. 3a) and spirals ($\chi^2_1 = 1.23$, $P = 0.26$; Fig. 3b); the same pattern was observed in C. morretes webs (radii: $\chi^2_1 = 0.78$, $P = 0.37$; Fig. 3c; spirals: $\chi^2_1 = 0.20$, $P = 0.65$; Fig. 3d).

Pupae Survivorship in Unmodified and Cocoon Webs

For the 13 third-stage larvae that were reintroduced to the same cocoon web of their C. fililineata host, we recorded the emergence of nine adults and four mortalities. Transfer of 15 third-stage larvae from their original cocoon web to a normal C. fililineata web resulted in emergence of three adults and 12 mortalities. Fifteen third-stage larvae were reintroduced to the same cocoon web of their host C. morretes; we observed the emergence of six adults and nine mortalities. The transfer of 15 third-stage larvae from the original C. morretes cocoon web to an unmodified host web resulted in the emergence of one adult and 14 mortalities.

Survivorship of parasitoid individuals reintroduced to the same cocoon web was higher than that of parasitoid larvae transferred from the original cocoon web to an unmodified web (survival analysis: C. fililineata: $\chi^2_{25} = 6.29$, P = 0.012; Fig. 4a; C. morretes: $\chi^2_{27} = 6.41$, P = 0.011; Fig. 4b). Also, parasitoid survivorship was not influenced by rain volume (survival analysis: C. fililineata: P = 0.37; C. morretes: P = 0.09).

A summary observation of both spider species showed the following pupal mortality factors: (1) web rupture due to rain (independent of rain intensity); cocoons fell to the ground and were subsequently preyed upon by an indeterminate predator (10.3% cocoon webs, 39.6% normal webs); (2) web rupture due to falling branches (3.4% cocoon webs); (3) predation by Mimetidae spiders (3.4% cocoon webs, 1.7% normal webs); and (4) ant predation occurring only when the web was partially broken and the cocoon came into contact with the vegetation, allowing ants access (5.1% cocoon webs, 3.4% normal webs).

DISCUSSION

Our results show that behavioural modifications of parasitized C. fililineata and C. morretes increases parasitoid polysphinctine wasp survival. Results verified that P. sp. nr. purcelli and P. janzeni not only exploit host spiders as a nutritional resource, but also manipulate their hosts to reduce parasitoid mortality risk by predation and web rupture. We confirmed behavioural changes in the host spider (e.g. increased web resistance, particularly to rupture resulting from rain) triggered by the parasitoid on the host's last day of life.

Increased parasitoid pupae survival, demonstrated by the production of physical structures, satisfies Poulin's (1995) primary criterion: changes in orb web spider behaviour are adaptive to polysphinctine wasps. This evidence for the adaptive value of C. fililineata and C. morretes cocoon webs is also congruent with data reporting manipulation of host species that build structures to conceal pupae, including A. limbata (Matsumoto, 2009) and N. clavipes (Sobczak, 2013). In Cyclosa species, pupae remain exposed and protection is achieved solely by increased web resistance. These observations in species with distinct modified web architectures suggest that a convergent solution exists for similar selective pressures.

Modified webs of C. morretes and C. fililineata are constructed only by spiders parasitized by third-stage larvae, indicating that first- and second-stage larvae are unable to elicit behavioural changes in their hosts. Similar results were described for several spider species parasitized by polysphinctines (Eberhard, 2000a, 2010b; Korenko et al., 2014). As modified cocoon webs are ineffective in retaining insects and are thus not suitable traps, subtle change might be important to assure normal host feeding during parasitoid development from eggs to large third-stage larvae.

Increased resistance of modified webs of C. filililineata and C. morretes was likely due to a reduction in interception area and strengthening of web lines. Eberhard (2010a) suggested that orb reduction in cocoon webs, such as those observed here, decreased the chances of web rupture by preventing debris accumulation on the spirals. Spiral elimination might prevent interception by insects, which would otherwise result in web rupture and/or cocoon damage. Also, V radii (Fig. 1e) found in some C. morretes cocoon webs, probably improved the physical stability of the web and reduced the risk of web rupture due to natural events. V radii have been observed in cocoon webs of other host spider species, including Leucauge mariana (Tetragnathidae) parasitized by Hymenoepimecis tedfordi and Eruga ca. gutfreundi (Ichneumonidae) (Eberhard, 2013), Allocyclosa bifurca (Araneidae) parasitized by the wasp Polysphincta gutfreundi (Ichneumonidae) (Eberhard, 2010a), and Leucauge argyra, parasitized by Hymenoepimecis argyraphaga wasps (Ichneumonidae) (Eberhard, 2001). These studies suggest that V radii are integral structures for stability of modified webs with different architecture.

The maintenance of C. filililineata and C. morretes normal orb web design under starvation conditions differs from results of previous studies of orb web weavers (Baba & Miyashita, 2006; Mayntz et al., 2009; Vollrath & Samu, 1997). It is possible that the period of starvation corresponding to the larval development period is not long enough to induce changes in spider web building behaviour. Thus, we reject the hypothesis that food deprivation is responsible for web modification. This result is consistent with that observed for N. clavipes (Gonzaga et al., 2010), confirming that food restriction cannot explain behavioural modifications in parasitized spiders. Eberhard (2000a) proposed an alternative hypothesis, where substances secreted by the parasitoid larva into the host spider directly trigger web

modification. Additional evidence further supports this hypothesis: when parasitoid larvae were removed from host spiders after modified web construction, normal web design was progressively recovered in A. bifurca (Eberhard, 2010a), L. argyra (Eberhard, 2001) and N. clavipes (Sobczak, 2013), which suggests a dose-dependent mechanism.

Variation in cocoon web design, as we observed in C. morretes, was also identified in Anelosimus octavius (Theridiidae) parasitized by Zatypota solanoi (Ichneumonidae) (Eberhard, 2010b). In addition, Korenko et al. (2014) reported web modification variability among parasitoid Polysphincta and Sinarachna species (Ichneumonidae) on the same Araniella opisthographa (Araneidae) host. This variation might indicate that different chemicals induce spider behavioural modification, or that host responses to similar chemicals differ.

Several studies have indicated that, under specific conditions, nonparasitized spiders might construct web structures very similar to those of cocoon webs. Resting or moulting webs of Cyclosa oculata (Araneidae) (Zschokke & Bolzern, 2007), Theridion exevum (Theridiidae) (Weng & Barrantes, 2007), A. bifurca (Eberhard, 2010a), N. clavipes (Gonzaga et al., 2010), Argiope trifasciata (Araneidae), L. mariana (Eberhard, 2013), Cyclosa argenteoalba (Takasuka et al., 2015) and Tetragnatha montana (Korenko, Korenková, Satrapová, Hamouzová, & Belgers, 2015) resembled cocoon webs built by parasitized individuals of these same species. Also, Korenko and Pekár (2011) observed that nonparasitized Neottiura bimaculata (Theridiidae) spiders construct webs similar to modified webs in order to protect their eggsacs and to cover themselves during winter, and Theridion varians (Theridiidae) also constructs overwintering webs similar to modified webs of parasitized spiders. Finally, Gonzaga et al. (2015) described modified webs in

Leucauge volupis (Tetragnathidae), which included a structural element (barrier threads) present only in juvenile webs of the species. The results of our study indicate that behavioural manipulation of host spiders by parasitic polysphinctine wasps involves the same chemical substances that act during host moult, reproduction and hibernation. We conclude that understanding endocrine control in these three fundamental events shows promise in elucidating the mechanisms responsible for behavioural manipulation in host spiders.

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deposited in the arachnid collection of Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais (curator A. J. Santos), Minas Gerais, Brazil.

References

Anderson, J.F. (1974). Responses to starvation in the spiders Lycosa lenta Hentz and Filistata hibernalis (Hentz). Ecology, 55, 576–585.

Baba, Y., & Miyashita, T. (2006). Does individual internal state affect the presence of a barrier web in Argiope bruennichii (Araneae: Araneidae)? Journal of Ethology, 24, 75–78.

Crawley, M.J. (2013). The R book. (2nd ed.). London, U.K.: J. Wiley.

Eberhard, W.G. (2000a). Spider manipulation by a wasp larva. Nature, 406, 255–256.

Eberhard, W.G. (2000b). The natural history and behavior of Hymenoepimecis argyraphaga (Hymenoptera: Ichneumonidae) a parasitoid of Plesiometa argyra (Araneae, Tetragnathidae). Journal of Hymenoptera Research, 9, 220–240.

Eberhard, W.G. (2001). Under the influence: webs and building behavior of Plesiometa argyra (Araneae: Tetragnathidae) when parasitized by Hymenoepimecis argyraphaga (Hymenoptera: Ichneumonidae). Journal of Arachnology, 29, 354–366.

Eberhard, W.G. (2010a). Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. Animal Behaviour, 79, 375–383.

Eberhard, W.G. (2010b). New types of behavioral manipulation of host spiders by a parasitoid wasp. Psyche, 2010, 950614. http://dx.doi.org/10.1155/2010/950614.

Eberhard, W.G. (2013). The Polysphinctine wasps Acrotaphus tibialis, Eruga ca. gutfreundi, and Hymenoepimecis tedfordi (Hymenoptera, Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. Annals of the Entomological Society of America, 106, 652–660.

Gauld, I.D. (1991). The Ichneumonidae of Costa Rica. Gainesville, U.S.A: Memoirs of the American Entomological Institute.

Gauld, I.D., & Dubois, J. (2006). Phylogeny of the Polysphincta group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider ectoparasitoids. Systematic Entomology, 31, 529–564.

Gonzaga, M. O., Cardoso, J. C. F., & Vasconcellos-Neto, J. (2015). Do parasitoids explain differential abundance of two syntopic orb-weaver spiders (Araneae: Araneidae)? Acta Oecologica, 69, 113-120.

Gonzaga, M.O., Moura, R.R., Pêgo, P.T., Lee Bang, D., & Meira, F.A. (2015). Changes to web architecture of Leucauge volupis (Araneae: Tetragnathidae) induced by the parasitoid Hymenoepimecis jordanensis (Hymenoptera: Ichneumonidae). Behaviour, 152, 181–193.

Gonzaga, M.O., & Sobczak, J.F. (2007). Parasitoid-induced mortality of Araneus omnicolor (Araneae, Araneidae) by Hymenoepimecis sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. Naturwissenschaften, 94, 223–227.

Gonzaga, M.O., Sobczak, J.F., Penteado-Dias, A.M., & Eberhard, W.G. (2010). Modification of Nephila clavipes (Araneae: Nephilidae) webs by the parasitoids Hymenoepimecis bicolor and H. robertsae (Hymenoptera: Ichneumonidae). Ethology Ecology and Evolution, 22, 151–165.

Gonzaga, M.O., & Vasconcellos-Neto, J. (2012). Variation in the Stabilimenta of Cyclosa fililineata Hingston, 1932, and Cyclosa morretes Levi, 1999 (Araneae:

Araneidae), in Southeastern Brazil. Psyche, 2012, 396594. http://dx.doi.org:10.1155/2012/396594.

Higgins, L., & Goodnight, C. (2010) Nephila clavipes females have accelerating dietary requirements. Journal of Arachnology, 38, 150–152.

Jensen, K., Mayntz, D., Toft, S., Raubenheimer, D., & Simpson, S.J. (2011). Nutrient regulation in a predator, the wolf spider Pardosa prativaga. Animal Behaviour, 81, 993–999.

Korenko, S., Isaia, M., Satrapová, J., & Pekár, S. (2014). Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecological Entomology, 39, 30–38.

Korenko, S., Korenková, B., Satrapová, J., Hamouzová, K., & Belgers, D. (2015). Modification of Tetragnatha montana (Araneae, Tetragnathidae) web architecture induced by larva of the parasitoid Acrodactyla quadrisculpta (Hymenoptera, Ichneumonidae, Polysphincta genus-group). Zoological Studies, 54, 40. doi:10. 1186/s40555-015-0119-6.

Korenko, S., & Pekár, S. (2011). A parasitoid wasp induces overwintering behaviour in its spider host. PLoS One, 6(9), e24628. doi:10.1371/journal.pone.0024628.

Korenko, S., Satrapová, J., & Zwakhals, K. (2015). Manipulation of araneid spider web architecture by the polysphinctine parasitoid Zatypota picticollis (Hymenoptera: Ichneumonidae: Pimplinae). Entomological Science, 18, 383–388.

Levri, E. P. (1999) Parasite-induced change in host behavior of a freshwater snail: parasitic manipulation or byproduct of infection? Behavioral Ecology, 10, 234–241.

Matsumoto, R. (2009). ‘Veils’ against predators: modified web structure of a host spider induced by an ichneumonid parasitoid, Brachyzapus nikkoensis (Uchida) (Hymenoptera). Journal of Insect Behavior, 22, 39–48.

Matsumoto, R., & Konishi, K. (2007). Life histories of two ichneumonid parasitoids of Cyclosa octotuberculata (Araneae): Reclinervellus tuberculatus (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomological Science, 10, 267–278.

Mayntz, D., Toft, S., & Vollrath, F. (2009). Nutrient balance affects foraging behaviour of a trap-building predator. Biology Letters, 5, 735–738.

Nielsen, E. (1923). Contributions to the life history of the pimpline spider parasites (Polysphincta, Zaglyptus, Tromatobia) (Hym. Ichneum.). Entomologiske Meddelelser, 14, 137–205.

Poulin, R. (1995). ‘Adaptive’ change in the behaviour of parasitized animals: a critical review. International Journal for Parasitology, 25, 1371–1383.

Poulin, R. (2010). Parasite manipulation of host behavior: an update and frequently asked questions. In H. J. Brockmann (Ed.), Advances in the study of behaviour (pp. 151–186). Burlington, MA: Academic Press.

Schmid-Hempel, P. (2011). Evolutionary parasitology: The integrated study of infections, immunology, ecology, and genetics. Oxford, U.K: Oxford University Press.

Sobczak, J.F. (2013) Estudos biológicos e ecológicos da interação entre Nephila clavipes (Araneae, Nephilidae) e o parasitoide Hymenoepimecis bicolor (Hymenoptera, Ichneumonidae, Pimplinae). (Doctoral thesis). São Carlos, SP, Brazil: Universidade Federal de São Carlos.

Takasuka, K., Yasui, T., Ishigami, T., Nakata, K., Matsumoto, R., Ikeda, K., et al. (2015). Host manipulation by an ichneumonid spider ectoparasitoid that takes advantage of preprogrammed web-building behaviour for its cocoon protection. Journal of Experimental Biology, 218, 2326–2332.

Thomas, F., Adamo, S., & Moore, J. (2005). Parasitic manipulation: where are we and where should we go? Behavioural Processes, 68, 185–199.

Velozo, H.P. & Goés-Filho, L. (1982). Fitogeografia brasileira; classificação fisionômica-ecológica. Ecologia da vegetação neotropical. Boletim Técnico do Projeto RADAMBRASIL, 1, 1–80.

Vollrath, F., & Samu, F. (1997). The effect of starvation on web geometry in an orb-weaving spider. Bulletin of the British Arachnological Society, 10, 295–298.

Weng, J. L., & Barrantes, G. (2007). Natural history and larval behavior of the parasitoid Zatypota petronae (Hymenoptera: Ichneumonidae). Journal of Hymenoptera Research, 16, 326–335.

World Spider Catalog (2015). World spider catalog (Version 16). Bern, Switzerland: Natural History Museum of Bern. http://wsc.nmbe.ch.

Yu, D.S., Horstmann, K., & Van Achterberg, K. (2012). Taxapad. http://www.taxapad.com/contact.php.

Zschokke, S., & Bolzern, A. (2007). Erste Nachweise sowie Kenntnisse zur Biologie von Cyclosa oculata (Araneae: Araneidae) in der Schweiz. Arachnologische Mitteilungen, 33, 11–17.

Zschokke, S., & Herberstein, M.E. (2005). Laboratory methods for maintaining and studying web-building spiders. Journal of Arachnology, 33, 205–213.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). Mixed effects models and extensions in ecology with R. New York, U.S.A.: Springer Press.

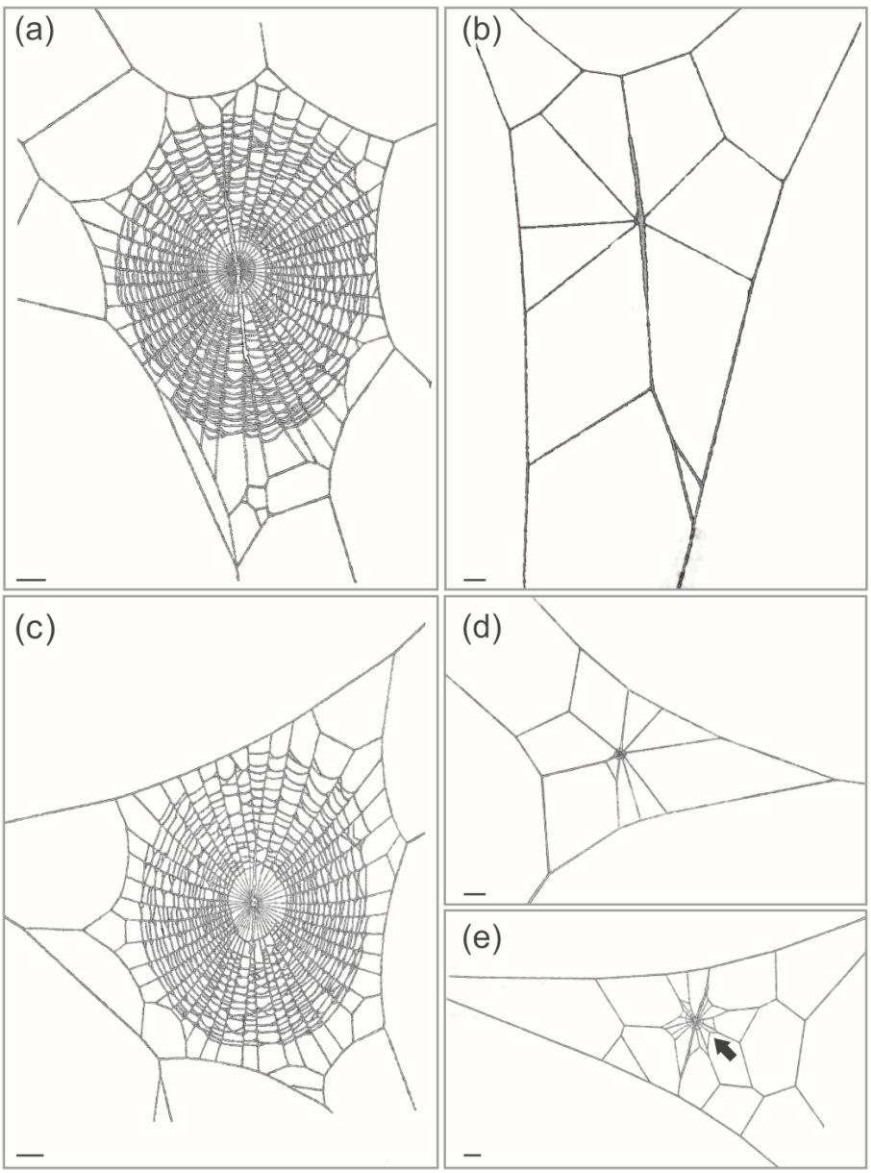

Figure 1. Cyclosa fililineata and C. morretes webs. (a) Normal web of C. fililineata. (b) Cocoon web of C. fililineata parasitized by Polysphincta sp. nr. purcelli. (c) Normal web of C. morretes. (d) Most frequent form of C. morretes cocoon web parasitized by P. janzeni. (e) Least frequent form of C. morretes cocoon web parasitized by P. janzeni (arrow V radii). Scale bar: 1 cm.

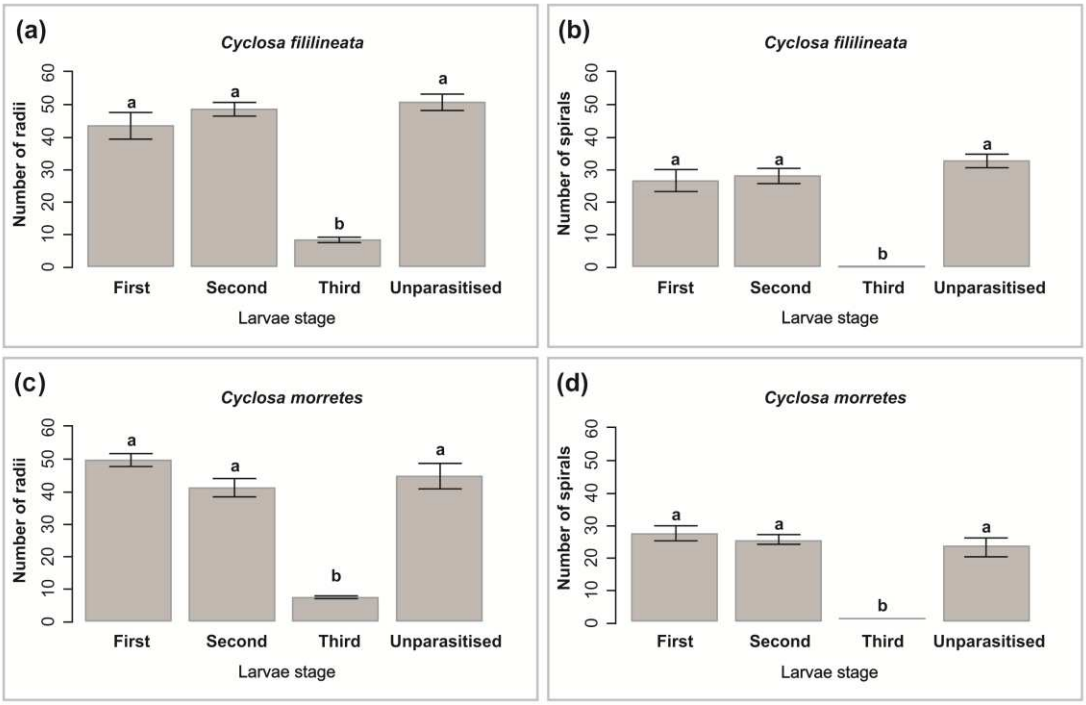

Figure 2. Web structure based on parasitoid larval stage. (a) Radii number modification in C. fililiineata webs. (b) Modification of sticky spiral loop number in C. fililiineata webs. (c) Radii number modification in C. morretes webs. (d) Modification of sticky spiral loop number in C. morretes webs. Different lowercase letters denote significant differences ($P < 0.05$) in web structure, evaluated through contrast analyses.

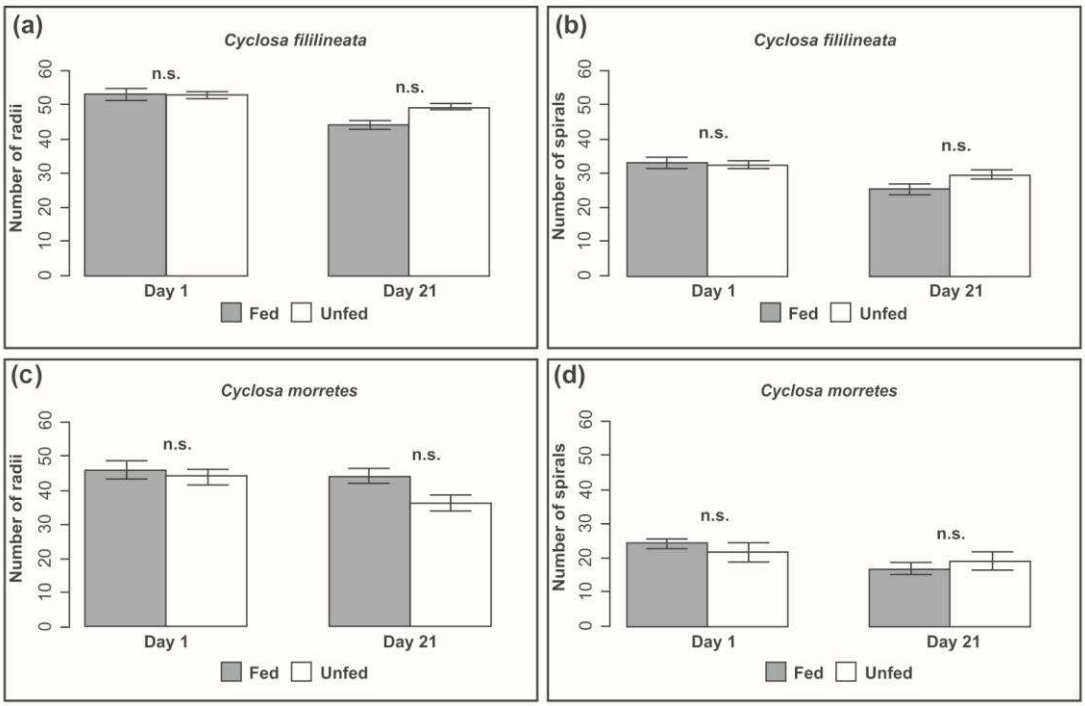

Figure 3. Web structure built by fed versus unfed individual hosts of C. fililineata and C. morretes. (a) Radii number in C. fililineata webs. (b) Sticky spiral loop number in C. fililineata webs. (c) Radii number in C. morretes webs. (d) Sticky spiral loop number in C. fililineata webs. NS denotes nonsignificant differences ($P \geq 0.05$) in web structure of fed (solid bars) versus unfed (open bars) spiders, evaluated through repeated measures ANOVA.

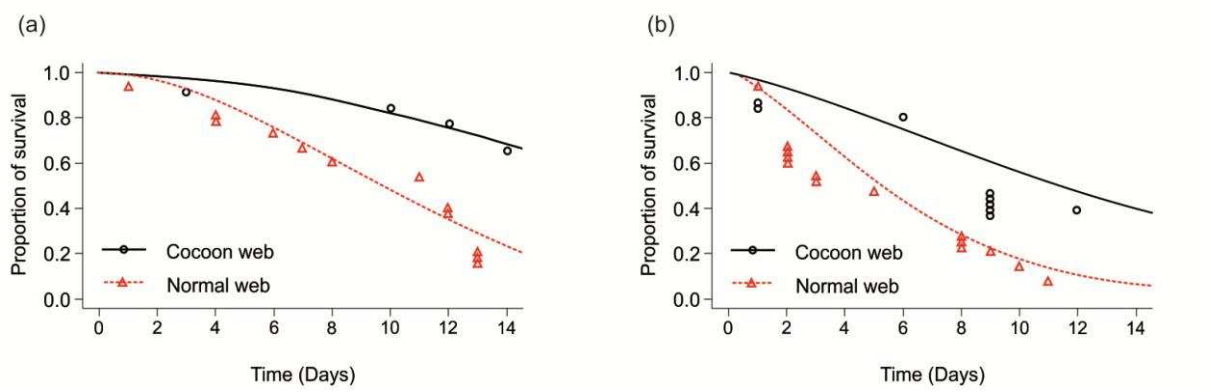

Figure 4. Parasitoid survivorship over time (days) on modified (cocoon) versus unmodified webs. (a) Survivorship of Polysphincta sp. nr. purcelli for C. fililineata host. (b) Survivorship of P. janzeni for C. morretes host. Superimposed points and triangles represent different samples with the same time to death.

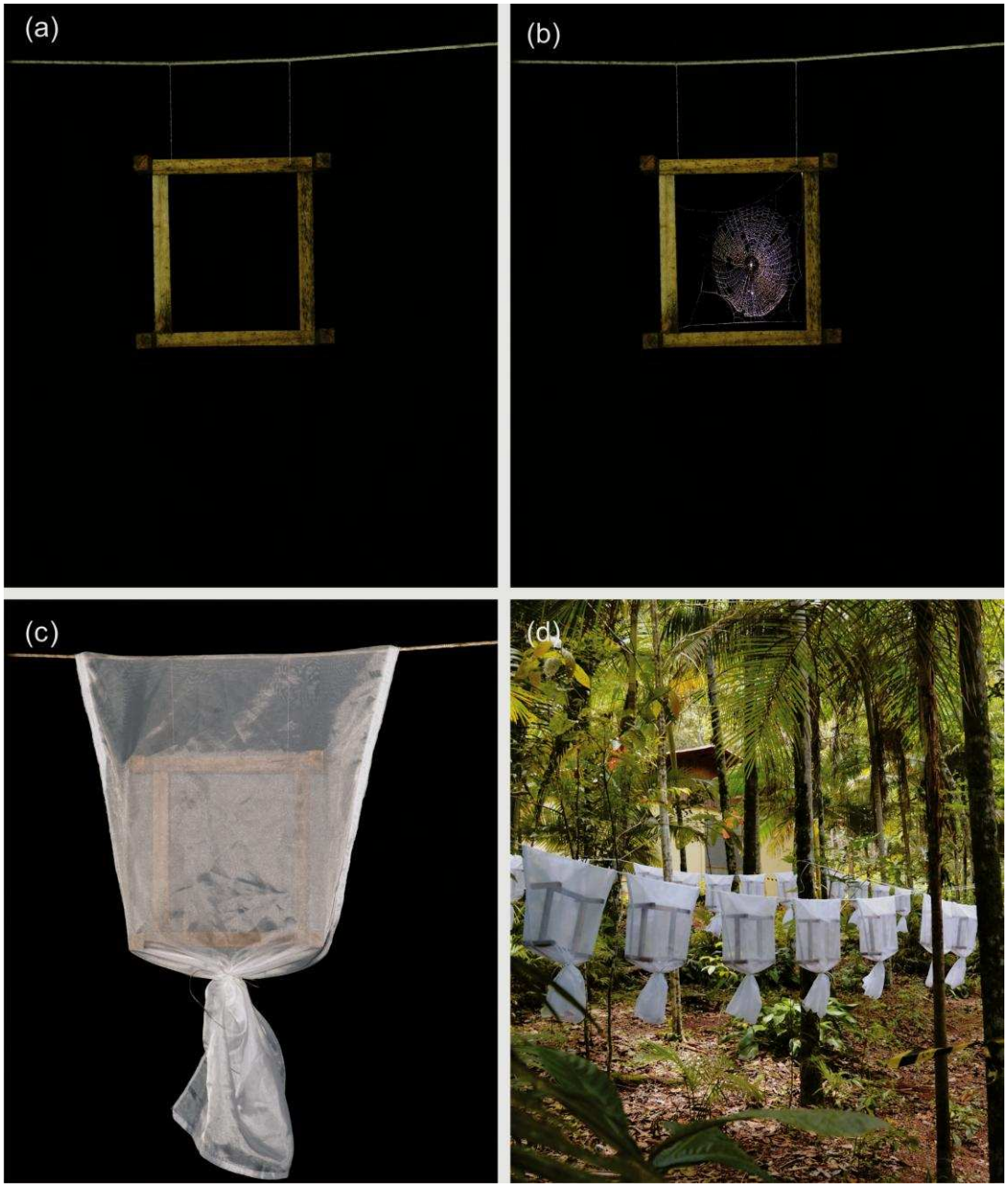
(a)
(b)
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Figure S1. Materials for food deprivation experiment. (a) Wooden frame (26 x 22 cm) suspended by a rope 1 m above ground level with two 15 cm long transverse wood pieces nailed to each frame corner to ensure the bag would not disrupt the spider web; (b) wooden frame (26 x 22 cm) with Cyclosa web; (c) frame/web bagged in a white organza fabric bag (80 x 50 cm) with a Velcro strip on the lateral side to facilitate opening and closing during food supply manipulation and photography and tied at the bottom to prevent prey entrance; (d) overview of food deprivation experiment in the field.

4 Capítulo três

4.1 The fountain of youth and insanity: on the mechanism of behavioral manipulation in orb-weaver spiders

This article will be submitted to Science (Report)

Title: The fountain of youth and insanity: on the mechanism of behavioral manipulation in orb- weaver spiders

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Abstract: Some ichneumonid wasps induce modifications in the web building behavior of their spider hosts to produce resistant "cocoon" webs. These structures hold and protect the wasp's cocoon during pupa development. The mechanisms responsible for host manipulation are unknown, but may depend on the concentration of psychotropic chemicals that the larva injects into the host. In addition, some spiders build cocoon webs similar to those normally built immediately before

ecdysis, suggesting that a molting hormone may be involved. Here, we report that Cyclosa spider species exhibiting behaviors presented higher 20-OH-ecdysone levels than parasitized spiders acting normally or unparasitized individuals. We suggest that the lack of control (or "insanity") that spiders have when constructing modified webs is triggered by anachronic activation of ecdysis.

One Sentence Summary: The mechanism how the behavior of two orb-weaving spider hosts is manipulated by their ichneumonid parasitoids is described.

Main Text: Parasites and parasitoids often alter specific behaviors of their hosts. Examples of changes range from subtle shifts in one aspect of the host's behavior to the performance of completely novel and complex behavioral patterns (1). Some of these changes may be primarily due to the host's own defensive responses to infection (1, 2) or to exploitation of host's nutritional resources by the parasites (3). Other changes in behavior are classified as host manipulation to acquire some benefit in survivorship or dispersal ability (1). In most cases of manipulation, little is known about the proximal mechanisms responsible for behavioral alterations (4).

Behavioral modifications of spiders by parasitoid wasps that form part of the Ephialtini group (5) (Hymenoptera: Ichneumonidae, Pimplinae) have been observed in several host families. The wasps lay one egg on the spider's abdomen, where the ectoparasitic larva develops by sucking hemolymph through holes perforated on the spider's cuticle. Usually, the host spider builds a modified web just before the wasp larva enters the final stage of development (6-12), but, in some cases, the number of radii and spirals of the orb web gradually decrease over several days before the

spider dies (13-15). After the spider builds this modified structure, the larva kills it and builds its cocoon attached to the silk threads spun by the host. Cocoon webs seem to be more resistant to rupture (12, 15, 16), with fewer components (e.g., radii and sticky spirals in orb webs) that are usually used for prey interception being present. Variations of this general pattern include the addition of barrier threads (17), shorter radii, doubled number of lines in each radius, the construction of a reinforced frame (13), or very fine and dense threads covering the spider and the parasitoid larva (18). These alterations are not a by-product of infection (14, 16), but seem to be directly induced by the parasitoid larva. This hypothesis is supported by the fact that removing the larva after the construction of the modified web results in the spider gradually rebuilding the web to its normal architecture (13, 15). The mechanism of manipulation may be related to the process of ecdysis because the modified webs of some host species resemble the molting webs spun by unparasitized immature individuals just before molting (12-14, this study – Figure 1).

The hormone 20-OH-ecdysone (20E) in spiders is responsible for renewing the old exoskeleton of juveniles (19). This knowledge led to the hypothesis that this hormone may elicit the behavioral responses of parasitized individuals that are under the influence of parasitoid larvae. Here, we show that Cyclosa morretes and Cyclosa fililineata (Araneidae) spiders carrying third-stage larvae of polysphinctine wasps (Ichneumonidae, Pimplinae) present higher levels of 20E (371 and 445 product ions, see Material and Methods) when compared with unparasitized spiders and spiders carrying second-stage larvae. The 20E product ions, 371 and 445, in spiders that showed modified behaviors were five times higher compared to those in the other groups (Figure 2, 3, 4). Also, 20E levels were similar for host spiders that built normal webs and the parasitoid larvae of both stages (Figure 2, 3, 4).

We suggest that the behavioral changes that occur in spiders carrying larvae close to their final stage of development are induced by increased 20E levels. This increase activates mechanisms that regulate web building changes that occur in juvenile spiders just before ecdysis. The injection of 20E or a precursor of mRNA responsible for 20E synthesis into the spider by the larvae may occur directly into the spider's hemolymph, while they are feeding on it. The production of the manipulative hormone by the parasitoid may only occur during the short specific interval of their development, close to the transition to the final stage of development. Thus, our observation that second and third stage larvae present low levels of 20E corresponds, respectively, to that of specimens before the production and after the inoculation of the hormone.

Similarities between cocoon and molting webs of both studied species include a reduction in number of radii and the absence of sticky spirals (Fig. 1). The same pattern was observed in Cyclosa argenteoalba (12) manipulated by Reclinervellus nielseni. In this case, a detailed comparison of web structures, building behavior, and silk spectral/tensile also showed that the few radii of both web types are decorated by many fibrous ultra violet-reflective threads. For Leucauge volupis (Tetragnathidae) attacked by Hymenoepimecis jordanensis, molting and cocoon webs share the presence of a three-dimensional component, a lower tangle, which may increase the stability of the horizontal web and is absent from normal webs constructed by adult spiders (17). In addition, the cocoon webs of Argiope trifasciata attacked by Acrotaphus tibialis include a three-dimensional structure similar to those observed in the molting webs of this species (20). Interestingly, it is also possible that the manipulation of spiders by another taxonomical group, Diptera larvae of Acroceridae, involves the same hormone. These endoparasites of spiders

also induce behavioral changes in web building behaviors that result in web designs similar to those of molting webs (21, 22).

However, the generality of this mechanism cannot be confirmed from the observation of cocoon webs of other spiders, such as Neottiura bimaculata and Theridion varians attacked by Zatypota percontatoria in the Czech Republic (10). The “cupula-like” structure built by parasitized T. varians and the denser web built by N. bimaculata are not similar to the structures constructed before molting. In fact, they resemble the web structures observed before overwintering in T. varians and before overwintering and oviposition in N. bimaculata. Thus, in these cases, behavioral modifications may be responses to the same substance produced and inoculated by the parasitoid larva (10). This substance may activate innate behaviors that also occur during the specific life-history periods of the hosts. Although ecdysteroids are also involved in the ovarian development of spiders (23), we would expect low levels of these hormones to be present before a period of reduced activity in cold winter conditions (19). Other cases of cocoon webs presenting unique characteristics have been reported for Allocyclosa (13), Anelosimus spp. (24), L. argyraphaga (8), Leucauge mariana (20) and L. roseosignatha (25). These characteristics of cocoon webs, differing from molting webs, show that other substances may be involved in the behavioral manipulation of spiders and that the mechanism described for Cyclosa species is not unique.

There is also variation in the way in which different parasitoid species modify the web design of the same host spider species. One such example is the case of Araniella opisthographa parasitized by Polysphincta and Sinarachna wasps (11). Although these differences may be related to different levels of 20E, it is possible that other the larvae produce other inducing substances alone or interacting with 20E,

leading to variation in web architecture. Alternatively, as suggested by Eberhard (13), it is possible that the action of the mature female wasp stabbing the host and injecting substances during or after the egg laying process may influence the sensitivity of the spider's nervous system, making it sensitive to the higher levels of 20E later injected by the larvae.

We conclude that the seeming lack of control (or "insanity") of parasitized C. morretes and C. fililineata is induced by the anachronic activation of mechanisms that were originally involved in changing the spider's exoskeleton before maturation. This phenomenon is achieved through increasing the levels of ecdysone during feeding by the parasitoid's larva. Thus, the exposition to ecdysone induces innate behaviors performed during the construction the simplified resistant web architectures that are used for molting. These modifications increase web stability and protection for the spider during periods of higher susceptibility to predators and prevent the risk of the web collapsing.

References and Notes:

1. R. Poulin, "[Parasite manipulation of host behavior: an update and frequently asked questions.]" in Advances in the study of behaviour, H. J. Brockmann, Ed. (Academic Press, Burlington, 2010), vol. 41, chap. 5.

2. F. Thomas, S. Adamo, J. Moore, Parasitic manipulation: where are we and where should we go? Behav. Process. **68**, 185-199 (2005).

3. I. Barber, D. Hoare, J. Krause. Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev. Fish Biol. Fisher. **10**, 131-165 (2000).

4. R. Poulin, F. Maure, Host manipulation by parasites: a look back before moving forward. Trends Parasitol. **31**, 563-570 (2015).

5. I. D. Gauld, J. Dubois, Phylogeny of the Polysphincta group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider ectoparasitoids. Syst. Entomol. **31**, 529-564 (2006).

6. E. Nielsen, Contributions to the life history of the Pimpline spider parasites (Polysphincta, Zaglyptus, Tromatobia) (Hym. Ichneum.). Entomol. Meddr. **14**, 137-205 (1923).

7. R. Matsumoto, K. Konishi, Life histories of two ichneumonid parasitoids of Cyclosa octotuberculata (Araneae): Reclinervellus tuberculatus (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomol. Sci. **10**, 267-278 (2007).

8. W. G. Eberhard, Spider manipulation by a wasp larva. Nature **406**, 255-256 (2000).

9. M. O. Gonzaga, J. F. Sobczak, Parasitoid-induced mortality of Araneus omnicolor (Araneae, Araneidae) by Hymenoepimecis sp. (Hymenoptera, Ichneumonidae) in southeastern Brazil. Naturwissenschaften **94**, 223–227 (2007).

10. S. Korenko, S. Pekár, A parasitoid wasp induces overwintering behaviour in its spider host. Plos One **6**, e24628 (2011).

11. S. Korenko, M. Isaia, J. Satrapová, S. Pekár, Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae). Ecol. Entomol. **22**, 30–38 (2014).

12. K. Takasuka, T. Yasui, T. Ishigami, K. Nakata, R. Matsumoto, K. Ikeda, K. Maeto, Host manipulation by an ichneumonid spider ectoparasitoid that takes

advantage of preprogrammed web-building behaviour for its cocoon protection. J. Exp. Biol. **218**, 2326-2332 (2015).

13. W. G. Eberhard, Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. Anim. Behav. **79**, 375-383 (2010).

14. M. O. Gonzaga, J. F. Sobczak, A. M. Penteado-Dias, W. G. Eberhard, Modification of Nephila clavipes (Araneae: Nephilidae) webs by the parasitoids Hymenoepimecis bicolor and H. robertsae (Hymenoptera: Ichneumonidae). Ethol. Ecol. Evol. **22**, 151-165 (2010).

15. J. F. Sobczak, "[Estudos biológicos e ecológicos da interação entre Nephila clavipes (Araneae, Nephilidae) e o parasitoide Hymenoepimecis bicolor (Hymenoptera, Ichneumonidae, Pimplinae)]", thesis, Universidade Federal de São Carlos, São Carlos, SP (2009).

16. T. G. Kloss, M. O. Gonzaga, J. A. M. Roxinol, C. F. Sperber, Host behavioural manipulation of two orb-weaver spiders by parasitoid wasps. Anim. Behav. **111**, 289-296 (2016).

17. M. O. Gonzaga, R. R. Moura, P. T. Pêgo, D. L. Bang, F. A. Meira, Changes to web architecture of Leucauge volupis (Araneae: Tetragnathidae) induced by the parasitoid Hymenoepimecis jordanensis (Hymenoptera: Ichneumonidae). Behaviour **152**, 181-193 (2015).

18. R. Matsumoto, 'Veils' against predators: modified web structure of a host spider induced by an ichneumonid parasitoid, Brachyzapus nikkoensis (Uchida) (Hymenoptera). J. Insect. Behav. **22**, 39-48 (2009).

19. J. C. Bonaric, "[Moulting Hormones]" in Ecophisiology of Spiders, W. Nentwig, Ed. (Springer-Verlag, Berlin, 1987), chap. 8.

20. W. G. Eberhard, The Polysphinctine wasps Acrotaphus tibialis, Eruga ca. gutfreundi, and Hymenoepimecis tedfordi (Hymenoptera, Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. Ann. Entomol. Soc. Am. **106**, 652-660 (2013).

21. E. I. Schlinger, "[The Biology of Acroceridae (Diptera): True Endoparasitoids of Spiders]" in Ecophisiology of Spiders, W. Nentwig, Ed. (Springer-Verlag, Berlin, 1987), chap. 24.

22. A. Cady, R. Leech, L. Sorkin, G. Stratton, M. Caldwell, Acrocerid (Insecta: Diptera) life histories, behaviors, host spiders (Arachnida: Araneida), and distribution records. Can. Entomol. **125**, 931-944 (1993).

23. M. Trabalon, A. M. Bautz, M. Moriniere, P. Porcheron, Ovarian development and correlated changes in hemolymphatic ecdysteroid levels in two spiders, Coelotes terrestris and Tegenaria domestica (Araneae, Agelenidae). Gen. Comp. Endocrinol. **88**, 128-136 (1992).

24. W. G. Eberhard, New types of behavioural manipulation of host spiders by a parasitoid wasp. Psyche 10.1155/2010/950614 (2010).

25. J. F. Sobczak, A. P. S. Loffredo, A. M. Penteado-Dias, M. O. Gonzaga, Two new species of Hymenoepimecis (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. J. Nat. Hist. **43**, 2691–2699 (2009).

26. H. W. Levi, The Neotropical and Mexican orb weavers of the genera Cyclosa and Allocyclosa (Araneae: Araneidae). Bull. Mus. Comp. Zool. **155**, 299-379 (1999).

27. The World Spider Catalog Association WSCA, “World Spider Catalog” (2015; www.wsc.nmbe.ch).

28. I. D. Gauld, The Ichneumonidae of Costa Rica. Mem. Am. Entomol. Inst. (Gainesville) **47**, 1-589 (1991).

29. M. O. Gonzaga, J. C. F. Cardoso, J. Vasconcellos-Neto, Do parasitoids explain differential abundance of two syntopic orb-weaver spiders (Araneae: Araneidae)? Acta Oecol. **69**, 113-120 (2015).

30. D. S. Yu, K. Horstmann, K. Van Achterberg, “Taxapad” (Home of Ichneumonoidea, 2012; www.taxapad.com/contact.php).

31. J. Zhou, Y. Qi, Y. Hou, J. Zhao, Y. Li, X. Xue, L. Wu, J. Zhang, F. Chen, Quantitative determination of juvenile hormone III and 20-hydroxyecdysone in queen larvae and drone pupae of Apis mellifera by ultrasonic-assisted extraction and liquid chromatography with electrospray ionization tandem mass spectrometry. J. Chromatogr. B **879**, 2533-2541 (2011).

32. J. Hikiba, M. H. Ogihara, M. Iga, K. Saito, Y. Fujimoto, M. Suzuki, H. Kataoka, Simultaneous quantification of individual intermediate steroids in silkworm ecdysone biosynthesis by liquid chromatography–tandem mass spectrometry with multiple reaction monitoring. J. Chromatogr. B **915**, 52-56 (2013).

33. A. F. Zuur, E. N. Ieno, N. J. Walker, A. A. Saveliev, G. M. Smith, Eds., Mixed effects models and extensions in ecology with R (Springer Press, New York, 2009).

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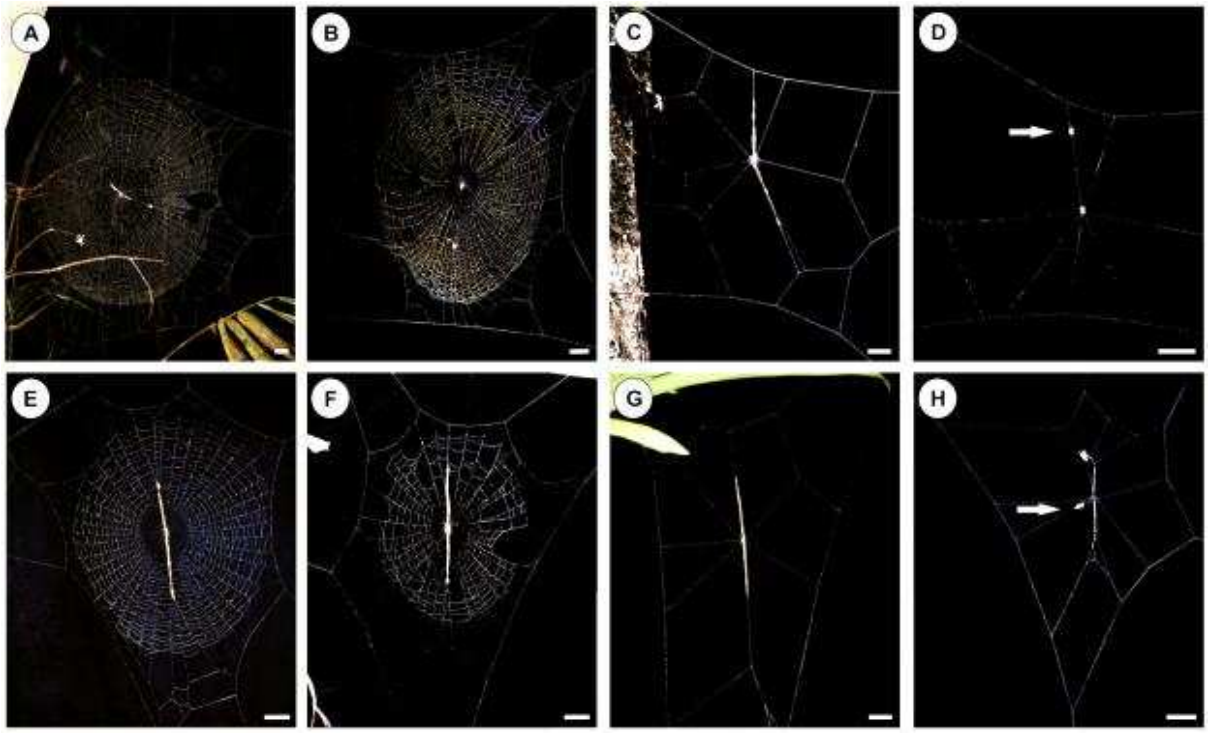

Fig. 1. Webs of Cyclosa morretes and Cyclosa fililineata. (**A**) web of an unparasitized C. morretes female; (**B**) web of C. morretes female parasitized by a second stage larva; (**C**) web built by C. morretes female parasitized by a third stage larva; (**D**) molting web of C. morretes (arrow old exoskeleton); (**E**) web of an unparasitized C. fililineata female; (**F**) web built by C. fililineata female parasitized by a second stage larva; (**G**) web built by C. fililineata female parasitized by third stage larva; (**H**) molting web of C. fililineata (arrow old exoskeleton). Scale bars: 1 cm.

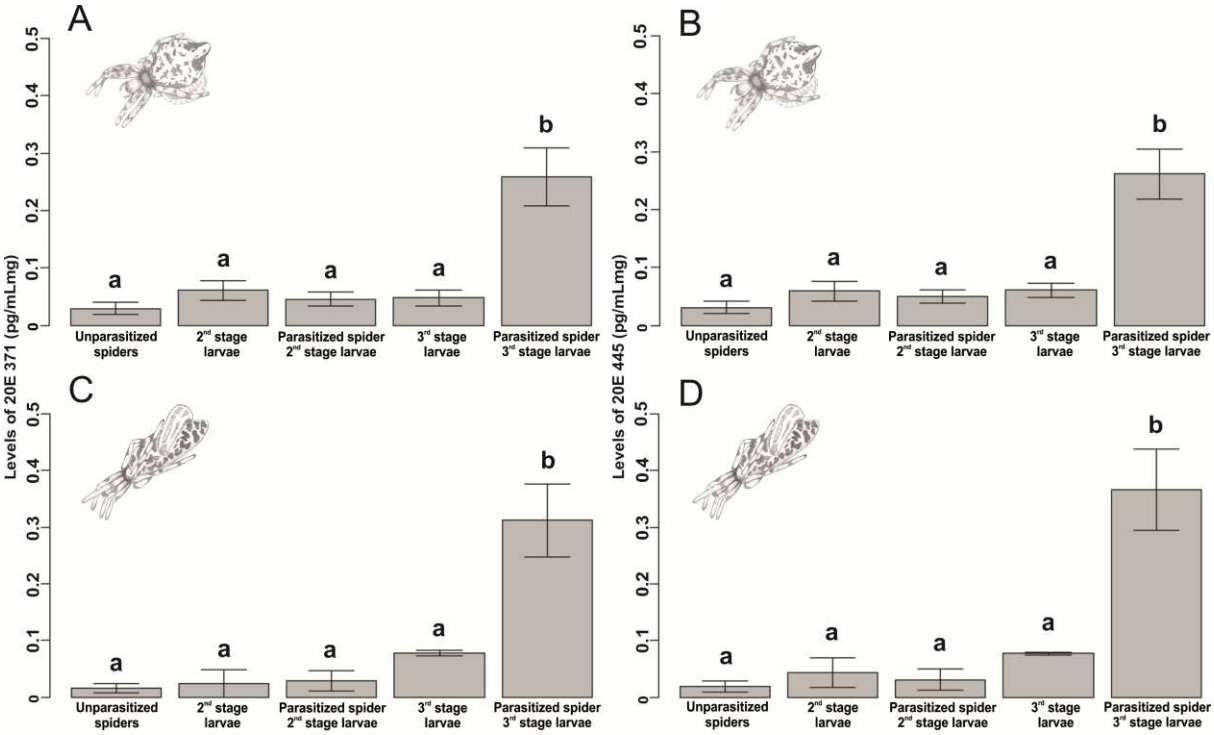

Fig. 2. Levels of 20-OH-ecdysone in parasitoid larvae and host spiders. (A) Levels of ecdysone (pg/mg) 371 ion product in unparasitized and parasitized Cyclosa morretes spider hosts, and in the respective Polysphincta janzeni parasitoid larva; (B) levels of ecdysone (pg/mg) 445 ion product in unparasitized and parasitized C. morretes spider hosts, and respective P. janzeni parasitoid larva; (C) levels of ecdysone (pg/mg) 371 ion product in unparasitized and parasitized Cyclosa fililineata spider hosts, and respective Polysphincta sp. nr. purcelli parasitoid larva; (D) levels of ecdysone (pg/mg) 445 ion product in unparasitized and parasitized Cyclosa fililineata spider hosts, and respective Polysphincta sp. nr. purcelli parasitoid larva; Different lower case letters correspond to significant differences ($P < 0.001$) in web structure, evaluated through contrast analyzes (A: $F(1,50) = 58.15$, $P < 0.001$; B: $F(1,50) = 72.95$, $P < 0.001$; C: $F(1,22) = 70.58$, $P < 0.001$; D: $F(1,22) = 82.32$, $P < 0.001$). Data from third stage larvae correspond to individuals obtained after the construction of modified cocoon webs by their hosts.

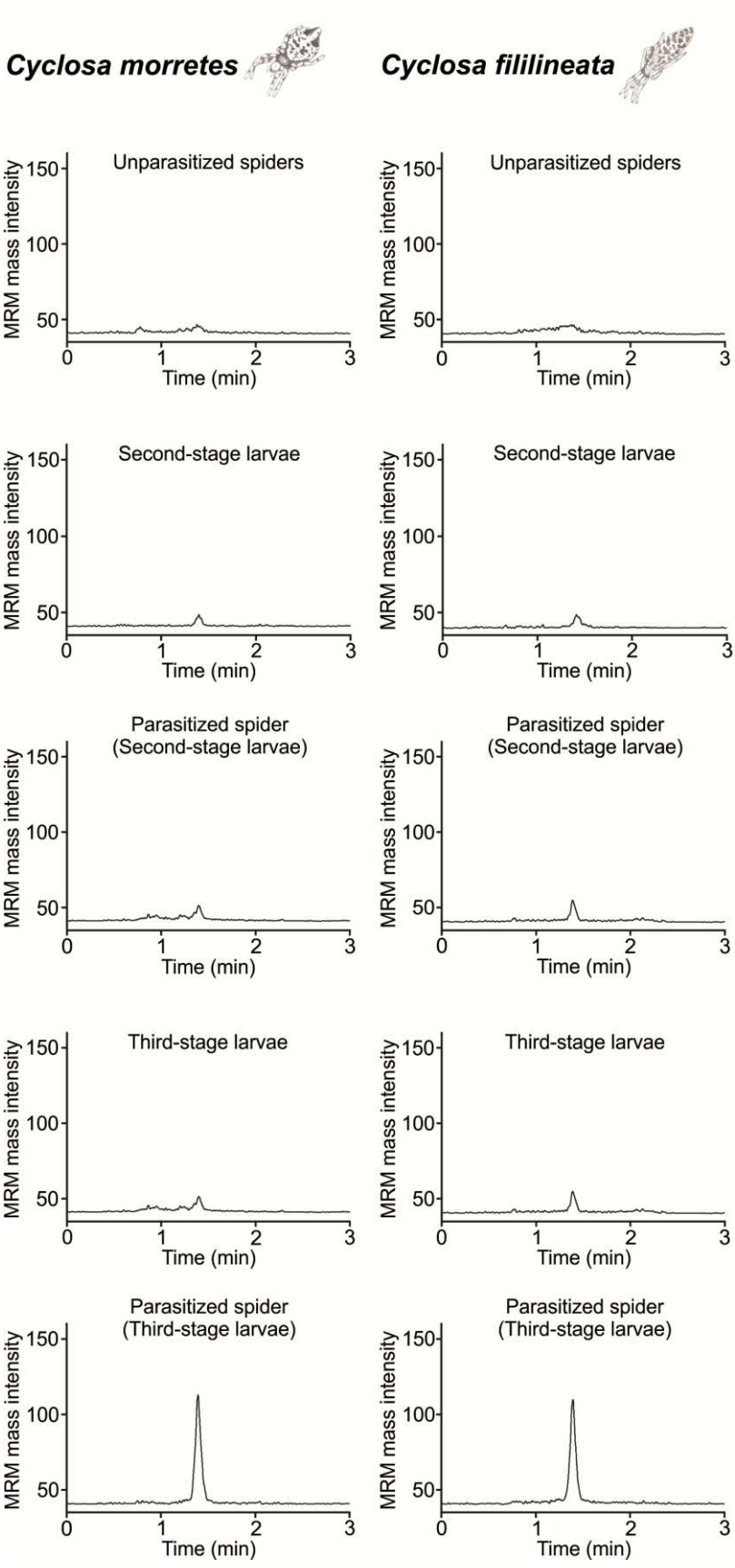
Cyclosa morretes
Cyclosa fililineata
Unparasitized spiders
Second-stage larvae
Parasitized spider
(Second-stage larvae)
Third-stage larvae
Parasitized spider
(Third-stage larvae)
MRM mass intensity
150
100
50
0
1
2
3
Time (min)

Fig. 3. Representative chromatograms of ecdysone 371 ion product in Cyclosa morretes and C. fililineata spider hosts and respective second or third stage parasitoid larva (Polysphincta janzeni and Polysphincta sp. nr. purcelli wasps, respectively). Chromatograms were observed by liquid chromatography mass spectrometry (LC-MS) with the multiple reaction monitoring (MRM) strategy detected with steroid-selective MRM channels. The steroids were separated by reversed phase LC with a quite complex gradient elution with acetonitrile/water. Data from third stage larvae correspond to individuals obtained after the construction of modified cocoon webs by their hosts.

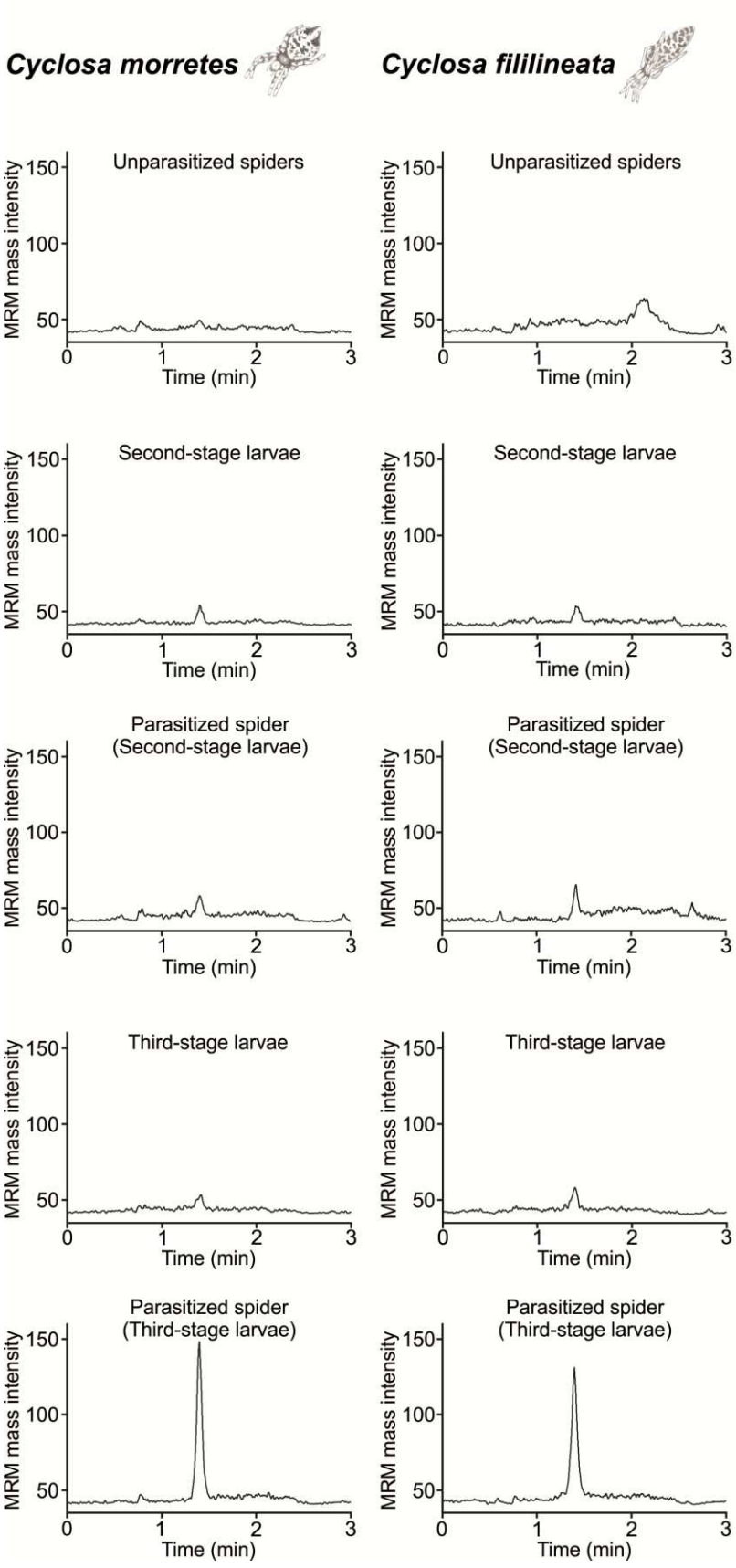
Cyclosa morretes
Cyclosa fililineata
Unparasitized spiders
Second-stage larvae
Parasitized spider
(Second-stage larvae)
Third-stage larvae
Parasitized spider
(Third-stage larvae)
MRM mass intensity
150
100
50
0
1
2
3
Time (min)

Fig.4. Representative chromatograms of ecdysone 445 ion product in Cyclosa morretes and C. fililineata spider hosts and respective second or third stage parasitoid larva (Polysphincta janzeni and Polysphincta sp. nr. purcelli wasps, respectively). Chromatograms were observed by liquid chromatography mass spectrometry (LC-MS) with the multiple reaction monitoring (MRM) strategy detected with steroid-selective MRM channels. The steroids were separated by reversed phase LC with a quite complex gradient elution with acetonitrile/water. Data from third stage larvae correspond to individuals obtained after the construction of modified cocoon webs by their hosts.

Supplementary Materials:

Materials and Methods

Databases S1 (separate file)

References (26-33)

Supplementary Materials:

Materials and Methods: Cyclosa fililineata is a small spider (total length of 4.7 mm) that ranges from Panama to Argentina. Cyclosa morretes is slightly larger (total length of 7.2 mm) and has only been detected in Brazil (26, 27). The parasitoid of C. morretes, Polysphincta janzeni, has been previously detected in Costa Rica (28) and the Brazilian Atlantic rainforest (29). The parasitoid of C. fililineata, Polysphincta sp. nr. purcelli, is probably a new species. The possibly closely related Polysphincta purcelli (Gauld, 1991) has been reported occur in Belize and Costa Rica (28, 30).

We collected 10 and 6 unparasitized females of C. morretes and C. fililineata, respectively. We also collected 11 and 5 females of C. morretes and C. fililineata, respectively, parasitized by second stage larvae of Polysphincta janzeni (C. morretes) and Polysphincta sp. nr. purcelli (C. fililineata). Finally, we collected 10 and 4 females of C. morretes and four of C. fililineata, respectively, parasitized by third stage larvae. We only collected females because males are rarely parasitized. All females of C. morretes were subadults, whereas all females of C. fililineata were adults that did not have egg sacs in their webs. Individuals of both spider species carrying third stage larvae were collected the day after they had constructed a cocoon web.

All spiders/larvae were collected during July 2014 and January 2015 from two Atlantic rainforest reserves located in Santa Teresa municipality, in Espírito Santo state, Brazil; specifically, Estação Biológica de Santa Lúcia (19º 57’ 56” S, 40º 32’ 24” W) and Reserva Biológica Augusto Ruschi (19º 54’ 26” S, 40º 33’ 11” W). Second-stage larvae were classified based on segmented bodies, while third-stage the

larvae had developed characteristic dorsal tubercles, which are absent in preceding developmental stages. Larvae were separated from their hosts in the laboratory of the Santa Lucia Station, after being collected and immediately frozen at 0 ºC in microtube vials. After separation, the spiders were transported frozen at 0 ºC to Núcleo de Análise de Biomoléculas of the Universidade Federal de Viçosa and kept at -20 ºC until the analyses.

To analyze the levels of 20-OH-ecdysone (20E) in spiders and larvae, we prepared stock solution of commercial 20E in methanol, at a final concentration of 5.0 ng/ml. The steroid 20E was obtained from Sigma-Aldrich (St. Louis, MO), with a purity of ≥93%. Methanol was obtained from Vetec Química Fina LTDA (Duque de Caxias, RJ, Brazil). The work standard solutions of 20E, with final concentrations of 0.1, 0.5, 0.8, 1.0, and 2 ng/ml, were prepared in methanol and stored at -20 ºC. The standards were used to construct the calibration curves by plotting the peak height versus 20E concentrations.

Spiders and larvae were weighed inside 2 mL microtubes. After weighing, 500 µl of methanol at ambient temperature was added to each microtube, and the samples were homogenized for 3 min using Tissue Master 125 (Omni International) to extract 20E. The sample was centrifuged at 11000 g for 20 min and the upper methanol layer was transferred to another microtube. Then, the samples were centrifuged again at 11000 g for 20 min and the upper methanol layer was transferred to injection glass vials and analyzed by liquid chromatography mass spectrometry (LC-MS).

The LC-MS ecdysone analyses were performed with the LC instrument UHPLC Agilent Technologies 1290 Infinity (Santa Clara, California, USA), used in combination with Agilent triple quadruple 6430 mass spectrometer with electrospray

ionization (ESI) (Santa Clara, California, USA) (adapted from 31, 32). The system operation was controlled and processed by the software MassHunter B06.00. Chromatographic separation was carried out using the Eclipse Plus C18um column (50 x 2.1mm) (Agilent Technologies, Santa Clara, California, USA). 20E was separated using binary gradient elution. Mobile phase A was water containing 0.01% acetic acid, and phase B was acetonitrile containing 0.02% acetic acid. Acetonitrile was obtained from Fluka Analytical (Seelze, Germany); acetic acid from Sigma-Aldrich (St. Louis, USA); and pure water was obtained by Milli-Q Direct Water Purification System (Darmstadt, Germany).

The gradient started with 15% phase B up to 1.5 min and then increased to 90% phase B, which was maintained for 6 min. Subsequently, phase B was decreased to 10% and maintained for 7 min. The total run time was 7 min, and an equilibration step of 1 min was included. The flow rate of the mobile phase and the column temperature were set at 0.3 mL/min (injection volume: 10µl) and 30 ºC, respectively. To avoid carryover, the auto sampler needle was rinsed automatically with the HPLC Flushing Solvent (Agilent Technologies, Santa Clara, California, USA) for 3 s before each analysis.

The mass spectrometry (MS) detection of the steroid was conducted by multiple reaction monitoring (MRM) in the positive ion mode. The tuning parameters optimized for 20E were gas at 300 ºC, drying gas flow at 3 L/min, nebulizer pressure at 15 psi, and Vcap voltage at 4000 V. The scan MS spectra were acquired by scanning the mass spectrometry in the m/z of 481, which is the precursor ion of 20E. The product ions obtained from this precursor were the ions 371 and 445 m/z in the retention time 1.4 (±5 s) min. The quantification of the 20E concentration (pg/ml) was obtained by comparing the peak height in each sample with calibration

curves. We only considered peaks with signal to-noise (S/N) ratios greater than five, as recommended by food and drug administration (FDA) guidelines.

We used ANOVA to compare 20E levels, using the 20E concentration (pg/ml)/sample weight (mg), among spiders parasitized by larvae of different stages and its respective larva, adjusting separate generalized linear models (GLM) for each response variable/spider species, using normal distributions. Significance was evaluated with contrast analyses, using F tests (33).

5 Conclusões Gerais

As modificações comportamentais das aranhas Cyclosa fililineata e Cyclosa morretes, induzidas por vespas Ephialtini, aumentam a sobrevivência dos parasitoides. Esse fato demonstra que essas modificações comportamentais são adaptativas para os parasitoides. Modificações comportamentais de hospedeiros, que aumentam o sucesso dos organismos parasíticos, também são observadas em outros grupos distantes, como as modificações comportamentais em lagartas Thyrinteina leucocerae, induzidas por parasitoides braconídeos, que aumentam a sobrevivência das pupas dos parasitoides. Isso evidencia que a solução evolutiva de manipular o hospedeiro por meio da indução de comportamentos que aumentam a proteção dos agentes manipuladores não é exclusiva da interação entre aranhas e vespas Ephialtini. A seleção dessa estratégia de manipulação em ancestrais filogeneticamente distintos possivelmente ocorreu frente a pressões seletivas semelhantes, relacionadas com a redução da probabilidade de morte das pupas dos parasitoides.

As modificações comportamentais induzidas pelas vespas em Cyclosa fililineata e Cyclosa morretes não são uma simples consequência do estado debilitado da aranha após ser sugada pela larva do parasitoide, mas o resultado do aumento no nível do hormônio ecdisona. Assim, as modificações das teias relacionadas à redução na área de impacto e aumento da resistência dos raios provavelmente foram favorecidas por reduzir a mortalidade das aranhas durante a ecdise, período no qual a aranha possui um exoesqueleto frágil e vulnerável a predadores. Dessa forma, as larvas dos parasitoides devem ativar esse mecanismo

inato de construção de teias de ecdise, o que resulta em maior sobrevivência dos parasitoides durante o estágio de pupa.

Apesar da dificuldade metodológica de induzir experimentalmente a alteração do comportamento das aranhas injetando 20-OH-ecdisona em seu corpo, encontrei evidências adicionais de que as larvas dos parasitoides devem produzir e transferir ecdisona para sua hospedeira, logo antes da alteração comportamental. Fiz análises (não incluídas no terceiro capítulo) de duas larvas de terceiro estágio de Polysphincta janzeni, que ainda não tinham induzido alterações comportamentais nas aranhas, e encontrei altos níveis de ecdisona nessas larvas, contrastando com os baixos níveis de ecdisona em larvas de terceiro estágio após a indução das alterações comportamentais, como apresentado no terceiro capítulo. Esse fato sugere que realmente as larvas de terceiro estágio de desenvolvimento podem armazenar e transferir diretamente 20-OH-ecdisona para as aranhas em um momento muito específico do desenvolvimento larval.

Apesar do mecanismo de manipulação comportamental com base em ecdisona poder explicar as alterações comportamentais em outras espécies de aranha, como Nephila clavipes (Tetragnathidae), Theridion exevum (Theridiidae), Cyclosa oculata e Cyclosa argenteoalba (Araneidae), certamente surgiram outras soluções evolutivas capazes de aumentar a sobrevivência dos parasitoides. Como discutido no capítulo três, em aranhas de clima temperado, a teia modificada difere das teias de ecdise, e são similares a teias de inverno e teias de reprodução. Assim, provavelmente o mecanismo responsável por induzir a manipulação em espécies de clima temperado é baseado em outras substâncias químicas, responsáveis por ativar o mecanismo inato das aranhas de construírem teias resistentes ao frio durante o inverno e durante períodos reprodutivos.

Além disso, teias modificadas por aranhas parasitadas não tem semelhança com nenhuma teia construída ao longo da vida dessas aranhas. Tais modificações incluem a inserção de fios de seda bloqueando a entrada das teias-funil modificadas de Agelana limbata ou padrões tridimensionais completamente novos das teias de indivíduos parasitados de Leucauge mariana. Esses padrões completamente novos de teias sugerem a existência de mecanismos relacionados com a produção de substâncias, quando parasitadas, não utilizadas pelas aranhas durante ecdise, reprodução ou períodos de modificações ambientais. Apesar de possíveis variações no mecanismo de manipulação comportamental de aranhas, a descoberta de um mecanismo com base na ativação de comportamentos inatos vai abrir um novo horizonte nas pesquisas sobre o tema, o que pode contribuir para a compreensão da evolução das interações entre parasitoides e hospedeiros.