

Evaluation of manipulative effects by an ichneumonid spider-ectoparasitoid larva upon an orb-weaving spider host (*Araneidae: Cyclosa argenteoalba*) by means of surgical removal and transplantation

Keizo Takasuka: Institute for Advanced Biosciences, Keio University, Mizukami 246-2, Kakuganji, Tsuruoka, Yamagata 997-0052, JAPAN; Graduate School of Agricultural Science, Kobe University, Rokkodai cho 1-1, Nada, Kobe, Hyogo 657-8501, JAPAN; E-mail: keizaf@gmail.com

Abstract. Surgical removal and larval transplantation experiments were carried out to evaluate behavioral manipulative effects caused by larvae of the polysphinctine ichneumonid, *Reclinervellus nielsenii* (Roman, 1923), on its orb-weaving spider host, *Cyclosa argenteoalba* Bösenberg & Strand, 1906 (Araneidae). Residual behavioral effects on spiders from which parasitoid larvae had been removed were recognized and are described. Some transplanted parasitoid larvae also successfully consumed alternative spider hosts provided to them, and completed metamorphosis. The removal experiment supports previous hypotheses proposed by Eberhard, that larval effects involve chemicals, which are still unidentified and are dosage-dependent, because (1) spiders with parasitoid larvae removed continued to show manipulated behavior, and (2) the later in their development that the parasitoid larvae were removed, the more acute remaining effects were (e.g., production of V radii, new hub loops and silk decorations on webs). Further, the results support the idea that the spider's web-building behavior is composed of independent units (modules), because whether or not specific web features were produced depended on when the larva was removed. Transplantation experiments provide a new technique for rearing parasitoid larvae whose host spider dies: penultimate instar parasitoid larvae were successfully reared following their removal from their original host by providing them with another host spider held by a mesh. Transplantation of a small young larva by gluing it onto another host that was released after successful transplantation, caused the new host to produce a modified web identical to the "cocoon" webs typically produced by parasitized spiders. This shows that venom injected by the adult parasitoid prior to oviposition is not necessary to induce behavioral changes in the host, which can be caused entirely by wasp larval secretions.

Keywords: Cocoon web, host web-manipulation, polysphinctine, *Reclinervellus nielsenii*, silk decorations

The larvae of many koinobiont parasitoids (i.e., those that allow hosts to be active post oviposition) are known to have manipulative and usurpative effects upon their living insect hosts (Adamo 1998, 2002; Brodeur & McNeil 1990; Brodeur & Vet 1994; Grosman et al. 2008) that contribute exclusively to the parasitoids' benefit. These are recognized as typical examples of the extended phenotype expressed in other organisms (Hughes et al. 2012; Mehlhorn 2015). Although it is one of the noticeable phenomena *in natura*, the mechanisms by which these manipulations are performed are usually difficult to unravel. For one thing, koinobiont parasitoids are generally endoparasites that may avoid physical problems such as dislodgement, desiccation and hosts' ecdysis (Gauld 1988). In other words, koinobiont ectoparasitoids are rare. Thus, evaluation of manipulative larval effects by means of removal of parasitoid larvae or by artificial transplantation to alternative hosts is seldom feasible. Developing such methods may provide an important breakthrough in understanding how host manipulations are achieved.

Spider web-building behavior can also be manipulated by ichneumonid koinobiont ectoparasitoids (i.e., the *Polysphincta* genus-group in Ichneumonidae). Currently the 258 species and 25 genera that make up the *Polysphincta* genus-group worldwide (Yu et al. 2016) constitute a monophyletic group (Gauld & Dubois 2006; Matsumoto 2016) with a wide host range as a whole, comprising about ten families of spiders. All polysphinctines are highly host-specific and some of them are known to manipulate host spiders causing them to weave a special reinforced and durable "cocoon web" in which parasitoid larval pupation occurs (Eberhard 2000b; Matsumoto 2009; Gonzaga et al. 2010; Korenko & Pekár 2011;

Takasuka et al. 2015). Their ectoparasitism would enable us to artificially remove or transplant parasitoid larvae from or onto spiders.

Polysphinctines are indeed the most successful experimental parasitoids, in that manipulative larval effects were evaluated using removal experiments. Eberhard (2000b, 2001, 2010) surgically removed polysphinctine larvae and observed lingering larval effects upon ex-host spiders using two polysphinctine species: *Hymenoepimecis argyraphaga* Gauld, 2000 upon *Leucauge argyra* (Walckenaer, 1841) (Tetragnathidae), which produces a horizontal orb web, and *Polysphincta gutfreundi* Gauld, 1991 upon *Allocyclosa bifurca* (McCook, 1887) (Araneidae) which makes a vertical orb web. He concluded that in both species pairs, the larval effects causing spiders' behavioral changes were caused chemically but not physically. He also showed that the effects were fast and long-lasting, though the effects were not permanent and hosts with parasitoid larvae removed slowly reverted to producing more normal orb webs (Eberhard 2001, 2010). Removal of parasitoid larvae at an early stage did not result in typical cocoon web construction suggesting either a chemical dosage effect or perhaps a change in the chemicals released by the parasitoid as it ages (Eberhard 2000b). In the case of *P. gutfreundi* on *A. bifurca*, the later the larvae were removed, the longer the spiders took to recover until normal orb web construction (Eberhard 2010).

These experiments provided many insights into how parasitoid larvae manipulate their host spiders. Here I present results of removal and transplant experiments with the polysphinctine, *Reclinervellus nielsenii* (Roman, 1923), which utilizes and manipulates *Cyclosa argenteoalba* Bösenberg &

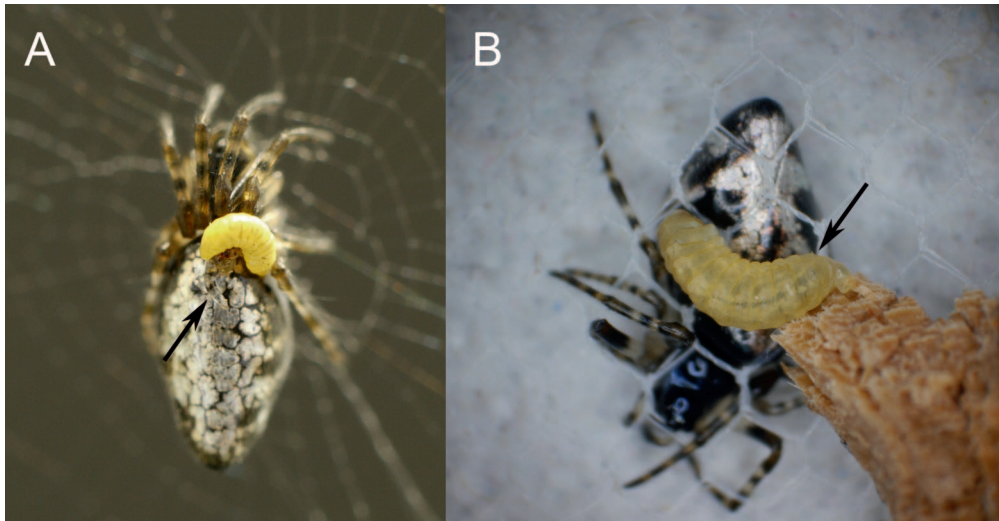


Figure 1.—Transplantation of *Reclinervellus nielsenii* larvae onto unparasitized *Cyclosa argenteoalba*: A. Transplantation of a small young larva onto a spider's abdomen with glue. Fixed glue (an arrow) which makes a junction between the larval saddle and the spider's abdomen can be seen under posterior (leftward) margin of the larva; B. Transplantation of a penultimate instar larva attached to a piece of wood onto a spider's abdomen which was kept immobile under the mesh. The glued point is indicated by an arrow but the glue is invisible from this angle.

Strand, 1906 (Araneidae) (Matsumoto & Konishi 2007; Takasuka et al. 2015). The transplant experiment, in which larvae were transferred to alternative host spiders (host replacement) provides a new technique to study subsequent larval development and the manipulative larval effects.

METHODS

Studied species and area.—*Cyclosa argenteoalba* is a vertical orb web weaver, characterized by a conspicuously silvery abdomen with black markings, the details of which vary

among individuals (Tanikawa 1992). *Reclinervellus nielsenii* attacks *C. argenteoalba* (see Figs. 2A, B), its main host in Japan (Matsumoto & Konishi 2007; Takasuka et al. 2015) but several other host species of *Cyclosa* are also known (Nielsen 1923; Fritzén 2005; Takasuka et al. 2017; Takasuka 2018). The adult wasp lays a single egg on an anterior margin of a spider's abdomen out of reach of spiders' legs. The larva of *R. nielsenii* sits transversely on the same part where the egg was located, allowing the host spider to continue normal life, i.e., koinobiont life style. The number of larval instars in another polysphinctine is thought to be three (Eberhard 2000a) but the

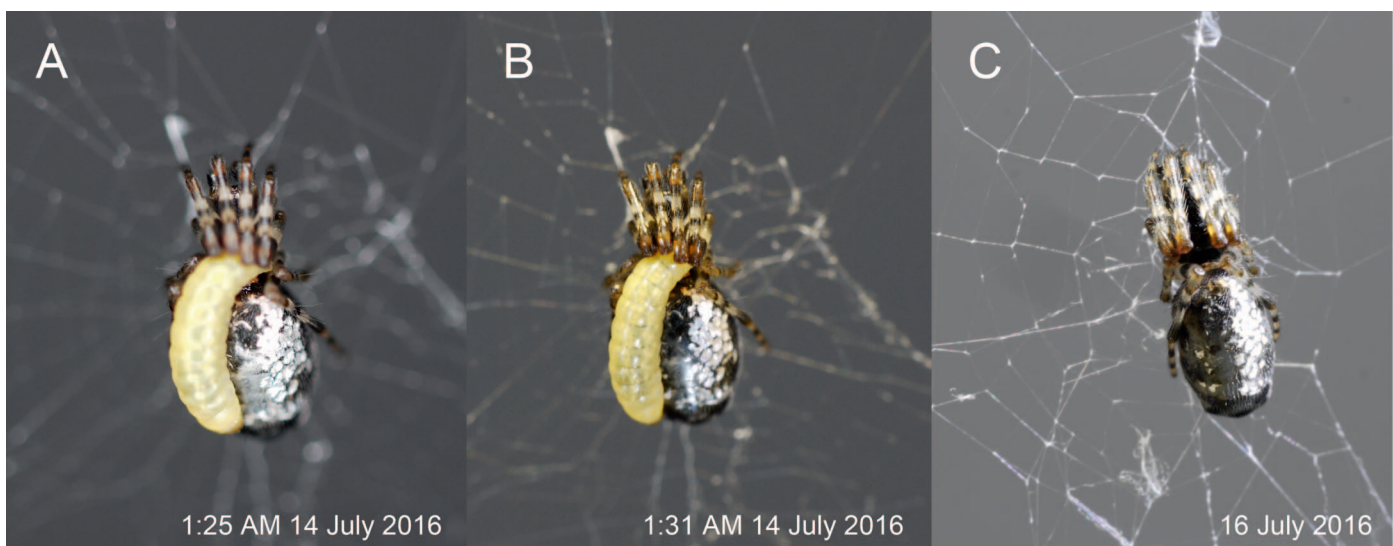


Figure 2.—Consumption of subcutaneous tissues of *Cyclosa argenteoalba* by a *Reclinervellus nielsenii* larva: A. A parasitized spider with a penultimate instar larva at 1:25 AM 14th July 2016 two hours before removal. Upper left silvery tissues were already consumed; B. Six minutes after A at 1:31 AM. Silvery tissues in left half of abdomen were consumed; C. Two days after larval removal (16th July). Consumed tissues had not recovered and no recovery was observed at all on 25th July as long as records remain. Newly woven hub loops can be seen in this figure.

precise number is still uncertain because skin-shedding is undetectable during middle instars.

The larvae of *R. nielsenii* attached to the abdomen of *C. argenteoalba* were collected in Kobe City (the campus of Kobe University) or the City of Tamba-Sasayama, Hyogo prefecture, Japan from April to May in 2015 and 2016. To obtain later instar larvae, parasitized spiders were reared in the laboratory following Takasuka et al. (2015).

Normal web-building behavior of *Cyclosa argenteoalba*.—Typical orb web consists of several elements such as frame threads, radial threads, sticky spiral and hub loops (Foelix 2010). Some characteristics of webs and building behavior of *C. argenteoalba* related to this study are described here.

Although the pattern of web radius construction *sensu* Eberhard (1982) of *C. argenteoalba* has not been ascertained, all *Cyclosa* spp. and also most of Araneidae spp. display Eberhard's pattern F1. The distinctive aspect of pattern F1 is that the spider breaks and reels up the provisional radius while laying the definitive radius on the way back to the hub (Eberhard 1982). A juvenile *C. argenteoalba* was observed to construct hub loops as a base of "temporal (scaffold) spiral" after all radius construction was done (unpublished data). In addition to the orb web, unparasitized *C. argenteoalba* sometimes construct simple webs putatively for molting, called the "resting (or molting) web;" these lack the sticky spiral but have conspicuous fluffy silk decorations (Takasuka et al. 2015). Although resting webs have hub loops, it is uncertain whether the hub loops are a vestigial structure of previous orb web or are newly constructed.

Host web-manipulation by *Reclinervellus nielsenii*.—Larvae of *R. nielsenii* at penultimate instar (see Figs. 2A, B; see also panel B in Supplementary Figure S1, online at <http://dx.doi.org/10.1636/JoA-S-18-082.S1>) manipulate *C. argenteoalba* to change the fine orb web into a simple and durable cocoon web with many of radii decorated with fibrous and fluffy silk decorations. Simplification of the web and the specific decorations conformed to those of a resting web constructed by unparasitized *C. argenteoalba*, suggesting that *R. nielsenii* exploits the pre-programmed behavior of the spider (Takasuka et al. 2015).

The manipulation process of *R. nielsenii* can be roughly categorized into three steps; (1) destruction of the sticky spirals (capture region) in the original orb web, (2) simplification of the former capture region by reducing the number of radii and including hub loop construction (see Results) and frame thread reinforcements, and finally, (3) decoration of radii (see supplementary movie 3 in Takasuka et al. 2015). Production of silk decorations was generally concentrated in the second half of the web-manipulation period and lasted until the eve of the spider's final resting on the hub (Takasuka et al. 2015), suggesting this is induced by a high dose of the manipulative inducer injected by a parasitoid larva. If the hub loop construction is an independent behavioral unit, it and silk decorations are thus advantageous to evaluate how acute the larval effects are because they are easily detected. Their expression indicates the level of the dosage-dependent manipulative larval effects that act beyond the certain threshold of web-manipulation.

In a similar web manipulation interaction, i.e., orb web simplification by *Polysphincta gutfreundi* against *Allocyclosa*

bifurca, the radii of cocoon webs often formed a 'V' shape (called "V radii") and thus the radius construction pattern was changed from F1 to F3 *sensu* Eberhard (1982) (Eberhard 2010). Thus, in this study V radii also are also considered an indicator of host manipulation by the parasitoid larva. The distinctive aspect of the F3 radius construction pattern is that the spider never breaks the provisional radius on the way back to the hub, and instead lays a second line as it goes, attaching it to the hub next to the starting point of the first line, resulting in V radii.

Verification of independence of hub loop construction in extra-orb webs.—To verify whether hub loops in resting webs and cocoon webs are a vestigial structure of previous orb web or independently constructed, direct observations on web-building behavior constructing both resting and cocoon webs was recorded with a digital single-lens camera, DMC-GH1 (Panasonic Corporation) and a macro lens (Leica DG Macro-Elmarit 45 mm/F2.8 Asph.).

Larval removal (remaining larval effects).—The polysphinctine larvae are attached to spiders' integument until their final instar by means of a "saddle" constructed under the 8th and 9th larval segments, which is formed from the parasitoid's egg chorion, coagulated spider blood and subsequent shed larval skins (Nielsen 1923; Eberhard 2000a). The saddle can be partly seen as it is at the connection point between a spider and a larva. Individual parasitized spiders were held under a microscope by a cotton (bottom) and a restraining mesh (top), and a protruding part of the larval saddle was picked up using superfine tweezers (SAKAKI DIF-104, M A Corporation) without disrupting the connection of the larva to its saddle. The saddle can be peeled off the spider's skin by several trials of tweezing, and it remains on the ventral side of the larva.

Parasitoid larvae were removed at two times during the penultimate instar: (1) immediately before manipulated behavior is exhibited by host spider (the expected day for initiation of manipulated behavior was judged from body size of the larvae; $n = 6$; Supplementary Figure S1 panel B); (2) after behavioral manipulation was initiated ($n = 5$; Supplementary Figure S1 panel C), as recognized by destruction of spirals (prey capture region). The former is regarded as an already-drugged spider prior to turning on the physiological switch responsible for manipulation of the host's behavior, while the latter is regarded as an already-enslaved web-weaver with the switch already on. The spiders with parasitoid larvae surgically removed were put back on their original web if present, or an empty rounded wire hoop. The shape and characteristics of subsequently constructed webs were observed every day until the spiders' recovery (as defined by construction of a normal orb web), molting or death, whichever occurred first.

Larval transplantation (host replacement).—Two types of transplantation were explored: (1) small young larvae ($n = 4$; Supplementary figure S1, panel A); (2) large penultimate instar larvae ($n = 6$; Supplementary Figure S1, panel B).

Different techniques had to be employed for the two ages of parasitoid larval transplants. A new host spider was restrained under a net mesh, and a tiny young larva was attached in place with glue (Ultra Tayoto SU, Konishi Co., Ltd) applied between the larval saddle and the spider's abdomen (Fig. 1A).

The spiders to which the parasitoid larva had been applied (abbreviated to 'Sp+P' derived from spider plus parasitoid) were released from the mesh and returned to the original web or to an empty rounded wire hoop, and then observed for larval effects upon alternative spiders.

Several difficulties were encountered during transplantation attempts: (1) the part that can be tweezed is limited to the larval saddle, where the glue is also placed, which makes getting the saddle off of the tweezers quite difficult; (2) the glue, which can only be used in a very small amount, hardens quickly, often before transplantation could be successfully carried out; and (3) unlike the polysphinctine larva alone, the glue was usually recognized by the spiders as a foreign object (Supplementary Video 1, online at <http://dx.doi.org/10.1636/JoA-S-18-082.S3>) and the attached larva was wiped off by the spider's legs, causing loss of the larva by the next day (sometimes spiders to which glue had been applied shed their skin with an attached larva one day after transplantation, seemingly in response to the glue attachment).

To show that the glue itself had no deleterious effects on spiders' behavior and physiology due to its potential toxicity, a negative control experiment was conducted. Six unparasitized spiders were treated as in the transplantation experiment with the same glue in nearly the same amount on their dorsum (See panel A in Supplementary Figure S2, online at <http://dx.doi.org/10.1636/JoA-S-18-082.S2>) and released onto individual, empty wire hoops to observe the effects of the glue.

The transplantation technique for small parasitoid larvae could not be applied to penultimate instars because they were too large to smoothly pass through the mesh used to restrain the spider. Further, the larvae made strong movements which prevented glue attachment to the spider's abdomen. Therefore, the larval saddle of the penultimate instar was glued to the tip of a thin piece of wood, and the wood was positioned so the larva's head could contact the abdomen of a spider that was held immobile under the mesh throughout the experiment (Fig. 1B). In these experiments, only the growth of the parasitoid larva was observed, not effects of the larva on the new spider host.

RESULTS

Verification of independence of hub loop construction in extra-orb webs.—A juvenile *C. argenteoalba* was observed to newly produce hub loops during resting web construction (Supplementary Video 2, online at <http://dx.doi.org/10.1636/JoA-S-18-082.S4>). Hub loops of a cocoon web were also observed to be newly produced by a manipulated spider (Supplementary Video 3, online at <http://dx.doi.org/10.1636/JoA-S-18-082.S5>) in the course of web simplification.

Larval removal (remaining larval effects).—Table 1 summarizes the effects of parasitoid larvae that remained following larval removal. Removed penultimate instar larvae had started to consume the silvery subcutaneous tissues of the host spider (Figs. 2A, B) and the silvery tissues were not replaced after removal (Fig. 2C).

Five spiders from which parasitoid larvae were removed (abbreviated to 'Sp-P' derived from spider minus parasitoid) immediately before the manipulated behavior was exhibited (abbreviated to 'Sp-P Bef') went on to display more or less manipulated behaviors, especially the destruction of the

previous spiral, the initial step of manipulation. However, it should be noted that spiral destruction alone could be residual manipulative effects of the removed larva, or a routine part of usual preparation for building the next orb; these two possibilities cannot be distinguished.

Two spiders from which parasitoid larvae were removed only showed destruction of the capture region and then immediately constructed normal orb webs one day after parasitoid removal, though one of these produced an obviously decreased number of spirals (Fig. 3B) in comparison to the orb web constructed before larval removal (Fig. 3A).

Three other spiders constructed simplified webs consisting of several radii, including several V radii, with new hub loops—the second step of manipulation. Two of these had silk decorations on their radii (Fig. 4), the final step of manipulation. Although one had only one radius decorated, another showed the strongest effects on its web and molted two days after the modified web construction and before normal web construction. All five spiders finally constructed normal orb webs.

In contrast, all Sp-P after the initiation of manipulated behaviors (abbreviated to 'Sp-P Aft') showed more aspects of cocoon web-like construction than the previous group, building simplified webs with V radii, new hub loops and silk decorations (Fig. 5). However, all but one of these Sp-P Aft finally did construct normal orb webs (one had no record of orb web construction but molted).

Larval transplantation (host replacement).—All six penultimate instar larvae succeeded in making cocoons and pupating after devouring the new host spider that was provided. All but one of these emerged as adult wasps; one individual died before emergence.

Three out of four transplantations of small young larvae failed, two of these because the transplanted parasitoid larva was removed by the host spider. However, one parasitoid larva which was transplanted on 5 June 2015 completed its growth on 12 June while the Sp+P kept building an orb web and successfully feeding (Supplementary Video 4, online at <http://dx.doi.org/10.1636/JoA-S-18-082.S6>). Web-manipulation occurred immediately before the Sp+P was killed by the transplanted parasitoid larva (Supplementary Videos 5, online at <http://dx.doi.org/10.1636/JoA-S-18-082.S7>). The cocoon web bore hub loops and silk decorations on its radii (Fig. 6). This specific, previously recorded decorating behavior (i.e., alternate pulling movements with legs IV as in prey wrapping) of the manipulated Sp+P spinning silk decorations onto radii was also observed (Supplementary Videos 5). The parasitoid larva spun a cocoon on the hub of the cocoon web as usual and pupated but did not emerge, dying inside the cocoon.

In the negative control experiment, three of six spiders to which glue was applied constructed a normal orb web as usual, with the glue coagulating on their dorsum (see panel B in Supplementary Figure S2) three, five and eleven days after the glue application, respectively. Three other spiders neither died nor molted at least five days after the glue application and had survived the treatment. These indicate that the glue at least does not have a lethal or behavior-modifying effect on spiders.

Table 1.—Residual larval effects by *Reclinervellus nielsenii* upon web shape of *Cyclosa argenteoalba* from which parasitizing larva were removed (Sp-P) immediately before manipulated behavior is exhibited (Bef1-6) and after manipulation initiated (Aft1-5).

Individual	Date of removal	Release condition to which they were put back	Remaining effects and recovery process	Duration for recovery(days)
Bef1	2 May 2016	the original orb web	No conspicuous change in web shape. Dead on 9 May.	-
Bef2	4 May 2016	an empty wire hoop	Constructed a simplified but framed web from scratch with new hub loops and silk decorations (Fig. 4 and inset) on 5 May with several radii being V shape (V radii, Fig. 4 inset). Then molted on 7 May and finally constructed a normal orb web on 9 May.	3
Bef3	5 May 2016	the original orb web	Destroyed the spiral and made the web simplified consisting of several radii with new hub loops but no decoration with the spiral partly left on 6 May. One of the radii is V radii. Finally constructed a normal orb web on 9 May.	4
Bef4	5 May 2016	the original orb web	Destroyed the spiral and made the web simplified consisting of a few radii with new hub loops but with the spiral partly left on 6 May. One of the radii was decorated and another was recognized as V shape. Finally constructed a normal orb web on 10 May.	5
Bef5	9 May 2016	the original orb web	Destroyed the previous orb web and constructed a normal orb web on 10 May but obviously decreased the number of spiral loops from 41–42 (Fig. 3A) to 9–10 (Fig. 3B).	1
Bef6	9 May 2016	the original orb web	Destroyed the previous orb web and constructed a normal orb web on 10 May. Molted on 16 May.	1
Aft1	27 April 2016	an empty wire hoop	Constructed a simplified but framed web from scratch with new hub loops and silk decorations on 28 April with several radii being V shape. Then constructed a normal orb web on 29 April. Subsequently constructed a resting web with silk decorations and molted on 4 May.	2
Aft2	28 April 2016	an empty wire hoop	Constructed a simplified but framed web from scratch with half hub loops and silk decorations on 29 April and then increased radii with complete hub loops and increased silk decorations on 30 April (Fig. 5). Presence of V radii was unclear because of silk decorations. Subsequently constructed a normal orb web on 3 May.	5
Aft3	1 May 2016	an empty wire hoop	Constructed a simplified but framed web from scratch with complete hub loops and silk decorations on 2 May with several radii being V shape. Then constructed a normal orb web on 4 May.	4
Aft4	13 May 2016	the original orb web being modified (capture region already destroyed)	Continued web modification adding complete hub loops (Fig. 2C) and silk decorations on 15 May with several radii being V shape. Then constructed a normal orb web on 22 May.	9
Aft5	14 May 2016	the original orb web being modified (capture region already destroyed and frame lines repeatedly reinforced)	Continued web modification adding complete hub loops and silk decorations on 15 May with several radii being V shape and then molted on 19 May. No record of orb web construction.	5

DISCUSSION

By means of direct observation, it was demonstrated that hub loop construction is an independent unit in addition to the case of a base of temporary spiral in normal orb web construction. They are also not vestiges of hub loops of the

previous orb web, and are induced by behavioral manipulation by the parasitoid. The production of the hub loops in web-manipulation would help the wasp's cocoon to be attached vertically along the hub of the web.

Although this study involves a relatively small number of samples, it was shown that Sp-P continued manipulated web-

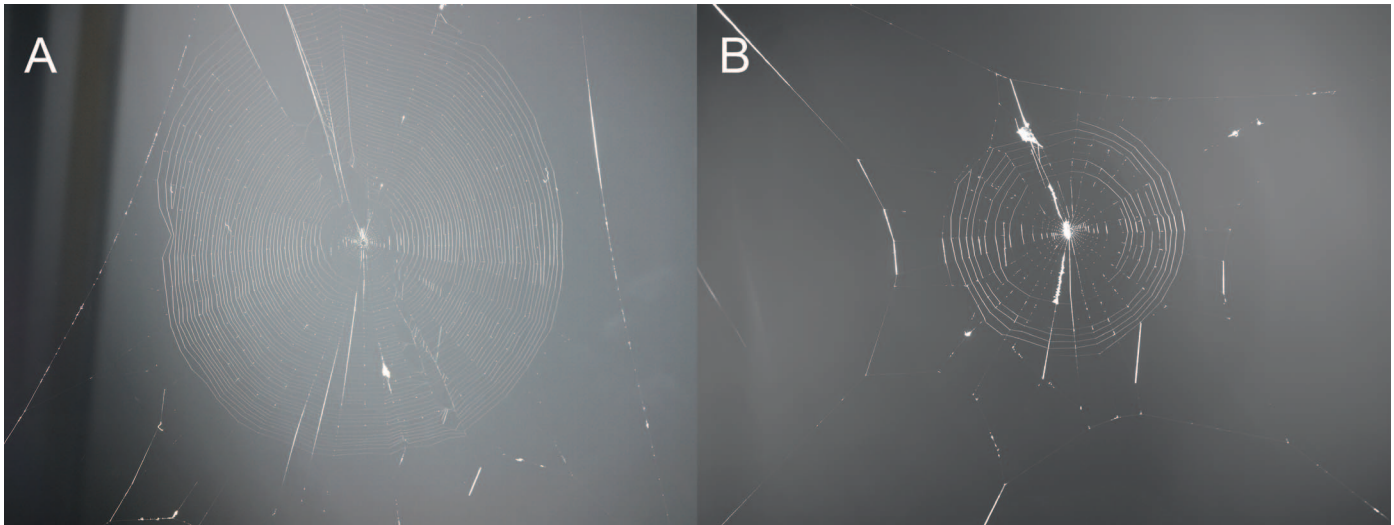


Figure 3.—Orb webs before and after larval removal, showing shallow effects brought by the parasitoid larva among the group (removal before manipulated behavior is exhibited, Sp-P Bef5): A. An orb web constructed by a parasitized spider before larval removal photographed on 9th May 2016; B. An orb web constructed by the ex-parasitized spider (same individual as A) photographed on 10th May. No modified web appeared but the web was diminished with extremely reduced numbers of spirals (41–42 vs. 9–10).

building behavior as described by Eberhard (2000b, 2001, 2010) for the orb-weavers *Leucauge argyra* and *Alloocylosa bifurca*. *Reclinervellus nielsenii* is thus the third polysphinctine genus and species for which it has been shown that manipulation continues after parasitoid larval removal.

In those more quantitative works with larger replicates than this study, Eberhard suggests three important implications

with regard to spider web-manipulation: (1) the changes in the spider's behavior must be induced chemically rather than by direct physical interference because the Sp-P continues to build the cocoon web even when the larva is removed shortly before manipulation would normally start (Eberhard 2000b, 2001, 2010); (2) the larval effects would be dosage-dependent because the later the larvae were removed, the more severe the



Figure 4.—A modified web constructed by an ex-parasitized spider, showing the strongest effects brought by the parasitoid larva among the group (removal before manipulated behavior is exhibited, Sp-P Bef2). The web has V radii (arrows in inset), hub loop and silk decorations.

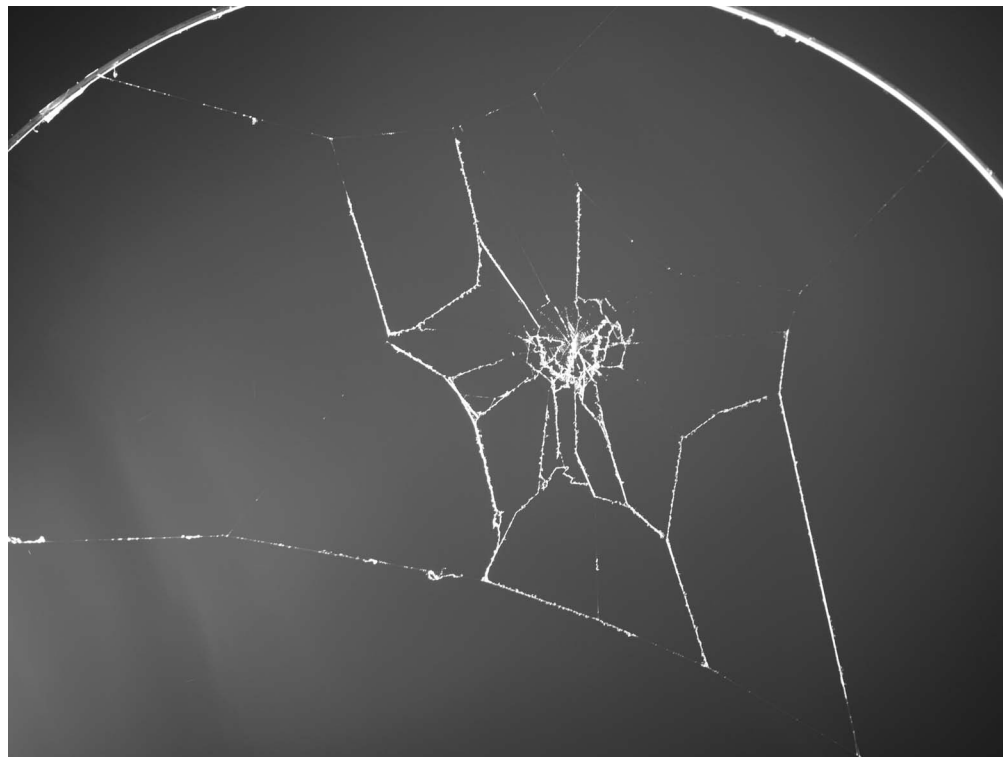


Figure 5.—Modified web constructed by an ex-parasitized spider from which parasitoid larvae were removed after manipulation initiated (Sp-P Aft2).

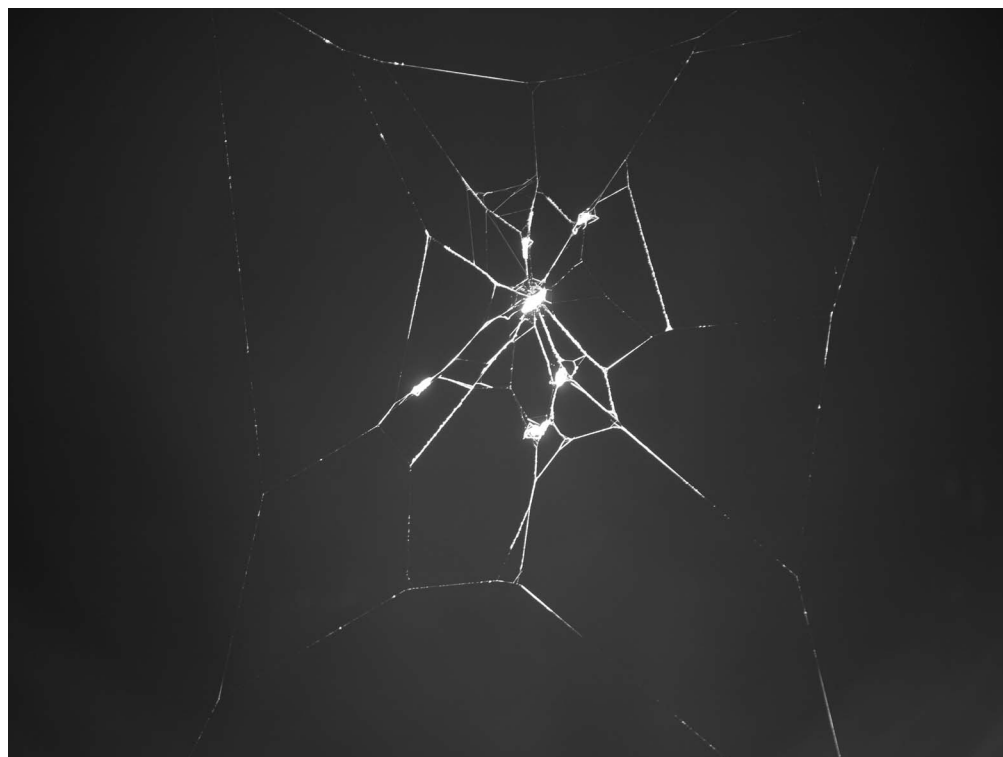


Figure 6.—Cocoon web constructed by a spider to which the young instar parasitoid larva had been applied (Sp+P) on its abdomen with glue.

effects on webs (Eberhard 2010); (3) spider's web-building behavior would be composed of independent units or modules because the larval effects induced specific web constructions independently (Eberhard 2000b, 2001). This study supports all these implications; (1) Sp-P (*Cyclosa argenteoalba*) continued to build a cocoon web; (2) remaining larval effects were more acute in removal after behavior manipulation was initiated than when the larvae were removed before manipulated behavior was exhibited (Table 1); (3) silk decorations were induced in Sp-P Aft while seldom induced in Sp-P Bef (Table 1). The same tendency observed in three different polysphinctine genera indicates that the mechanism of web-manipulation and even chemical composition of manipulative substance(s) could be similar.

The main aim of this study is the larval transplantation (host replacement). Transplantation of penultimate instar larvae was very effective, as all larvae completed devouring the new spider host that was provided to them, and all but one successfully emerged as adult wasps. This method can be applied to rearing of other polysphinctine larvae whose host spider accidentally dies. In order to observe larval effects, successful methods for the artificial attachment of penultimate instar larvae to new host spiders still need to be established. Although only one experimental transplantation of a small instar larva was successful, it induced web-manipulation in the new host spider, which constructed a complete cocoon web with silk decorations. There are several studies demonstrating venom injected by an ovipositing parasitoid chemically causes host behavioral manipulation both in aculeates (Libersat 2003; Gal et al. 2005; Gal & Libersat 2010) and 'Parasitica' (Shaw 1981, 1983). Eberhard (2010) did not rule out the possibility that specific portions of the spider's nervous system are rendered sensitive to products from the parasitoid larva by the venom delivered by the adult wasp. However, web-manipulation induced in the *C. argenteoalba* which had received no adult wasp venom shows conclusively that adult wasp venom is not necessary to induce web-manipulation. Thus, this research verified that web-manipulation can be brought about entirely by parasitoid larval secretions.

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SUPPLEMENTARY FILES

Supplementary Figure S1.—Stage definition of the larvae provided for experiments: (A) A small young larva; (B) A penultimate instar larva with a host spider not exhibiting manipulated behavior yet; (C) A penultimate instar larva with a host spider already exhibiting manipulated behavior.

Supplementary Figure S2.—Negative control experiment: (A) Newly applied glue on the dorsum of a unparasitized

spider held immobile under the mesh; (B) Same spider as A, constructing a normal orb web with the glue coagulating on their dorsum five days after the glue application. Arrows indicate applied glue.

Supplementary Video 1.—Wiping behavior of *Cyclosa argenteoalba* to which a *Reclinervellus nielseni* larva had been applied (Sp+P) on its abdomen with glue.

Supplementary Video 2.—Construction behavior of hub loop and silk decorations by an unparasitized *Cyclosa argenteoalba* building a resting web. The spider opened the hub of the hub loop by biting, and spun threads over the open area.

Supplementary Video 3.—Construction behavior of hub loop and silk decorations by a parasitized *Cyclosa argenteoalba* building a cocoon web under manipulation by a *Reclinervellus nielseni* larva.

Supplementary Video 4.—*Cyclosa argenteoalba* to which a *Reclinervellus nielseni* larva had been applied (Sp+P) eating a chironomid fly. Applied glue can be recognized.

Supplementary Video 5.—The entire sequence of cocoon web construction of *Cyclosa argenteoalba* (Sp+P) manipulated by the transplanted *Reclinervellus nielseni* larva. The video was speeded up by 20 times and scenes during which the spider is inactive and behavior is uncaptured were cut. The entire sequence of cocoon web construction in natural condition is available in Takasuka et al. (2015) (<http://movie.biologists.com/video/10.1242/jeb.122739/video-3>).

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