

RESEARCH ARTICLE

Host manipulation by an ichneumonid spider ectoparasitoid that takes advantage of preprogrammed web-building behaviour for its cocoon protection

Keizo Takasuka^{1,*}, Tomoki Yasui², Toru Ishigami², Kensuke Nakata³, Rikio Matsumoto⁴, Kenichi Ikeda⁵ and Kaoru Maeto¹

ABSTRACT

Host manipulation by parasites and parasitoids is a fascinating phenomenon within evolutionary ecology, representing an example of extended phenotypes. To elucidate the mechanism of host manipulation, revealing the origin and function of the invoked actions is essential. Our study focused on the ichneumonid spider ectoparasitoid *Reclinervellus nielsenii*, which turns its host spider (*Cyclosa argenteoalba*) into a drugged navvy, to modify the web structure into a more persistent cocoon web so that the wasp can pupate safely on this web after the spider's death. We focused on whether the cocoon web originated from the resting web that an unparasitized spider builds before moulting, by comparing web structures, building behaviour and silk spectral/tensile properties. We found that both resting and cocoon webs have reduced numbers of radii decorated by numerous fibrous threads and specific decorating behaviour was identical, suggesting that the cocoon web in this system has roots in the innate resting web and ecdysteroid-related components may be responsible for the manipulation. We also show that these decorations reflect UV light, possibly to prevent damage by flying web-destroyers such as birds or large insects. Furthermore, the tensile test revealed that the spider is induced to repeat certain behavioural steps in addition to resting web construction so that many more threads are laid down for web reinforcement.

KEY WORDS: Cocoon web, *Cyclosa argenteoalba*, Fibrous thread decoration (FTD), *Reclinervellus nielsenii*, Resting web, Tensile test

INTRODUCTION

Host manipulation, in which a parasitic agent chemically induces its host organism to express alternative behaviour (extended phenotype), occurs across a wide range of taxa and affects an array of ecological phenomena, such as population dynamics of both host and parasite, food chains and energy/material flow in ecosystems (Lefèvre et al., 2009). For instance, horsehair worms (Nematomorpha) manipulate their orthopteran intermediate hosts' behaviour, making them more accessible as food for trout (Sato et al., 2011) and Nematoda manipulate bumblebee queens, causing a reduction in nectar yield for non-host workers (Kadoya and Ishii,

2014). The studies of host manipulation from the point of view of parasites are also fundamental and invaluable to envisage such ecological dynamics. Lafferty and Shaw (2013) proposed that a change in host behaviour associated with parasitism, a positive link between altered host behaviour and parasite fitness, and mechanisms by which parasites alter host behaviour, are the three necessary factors in understanding how parasites manipulate their hosts.

Amongst the *Polysphincta* genus-group (informally, polysphinctines; Hymenoptera, Ichneumonidae), which are exclusively koinobiont spider ectoparasitoids, some members are well known to manipulate their host spiders to modify web construction (Eberhard, 2000, 2001, 2010a,b, 2013; Gonzaga and Sobczak, 2011; Gonzaga et al., 2010; Korenko et al., 2014; Korenko and Pekár, 2011; Matsumoto, 2009; Matsumoto and Konishi, 2007; Sobczak et al., 2009). Prior to killing the host spider, the parasitoid larva (penultimate instar) manipulates the host to build a specific and durable 'cocoon web' (*sensu* Eberhard, 2000), seemingly enhancing survival of parasitoid pupae, which depends on the host web's persistence against the elements or enemies.

This interaction between the polysphinctine and its host spider provides us with an opportunity to meet Lafferty and Shaw's (2013) demands: (1) behavioural change in the host spider is clearly exhibited in web-building steps and its product (i.e. cocoon web); (2) this system is ideal for comparing the functions between the unmanipulated (normal) web and the manipulated end product (cocoon web), thereby enabling us to quantitatively evaluate the fitness of the extended phenotype; (3) if the origin and resulting advantage of manipulated behaviour is quantitatively revealed, host manipulation mechanisms will come closer to being understood. In fact, the polysphinctine *Zatypota percontatoria* (Müller), which parasitizes several theridiid spider species, evokes the seemingly protective silk structure originally expressed only when the spider is overwintering (Korenko and Pekár, 2011), giving a clue as to host web-manipulation mechanisms within the wider polysphinctines but lacking their mechanical property analysis.

Reclinervellus nielsenii (Roman 1923) is a polysphinctine that parasitizes orb-weaving spiders (Fig. 1A): *Cyclosa argenteoalba* Bösenberg & Strand 1906 (Araneae, Araneidae; Fig. 1D inset) in Japan (Matsumoto and Konishi, 2007) and *Cyclosa conica* (Pallas 1772) in Europe (Fritzén, 2005; Nielsen, 1923). *Reclinervellus nielsenii* larvae seem to induce the host spider to modify its web form (Fig. 1C) into a simpler and seemingly more effective design (Fig. 1D) than the normal orb web to sustain the wasp's cocoon, both in *C. conica* (figs 9,10 in Nielsen, 1923) and *C. argenteoalba* (fig. 19 in Matsumoto and Konishi, 2007). Unparasitized healthy *C. argenteoalba* sometimes construct simple webs without the sticky spiral that we call a resting web (Fig. 1B), as also described for other orb-weaving spiders (Blackledge et al., 2011; Eberhard,

¹Laboratory of Insect Biodiversity and Ecosystem Science, Graduate School of Agricultural Science, Kobe University, Hyogo 657-8501, Japan. ²Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, Hyogo 657-8501, Japan. ³Faculty for the Study of Contemporary Society, Kyoto Women's University, Kyoto 605-8501, Japan. ⁴Osaka Museum of Natural History, Osaka 546-0034, Japan. ⁵Laboratory of Stress Cytology, Graduate School of Agricultural Science, Kobe University, Hyogo 657-8501, Japan.

*Author for correspondence (keizaf@gmail.com)

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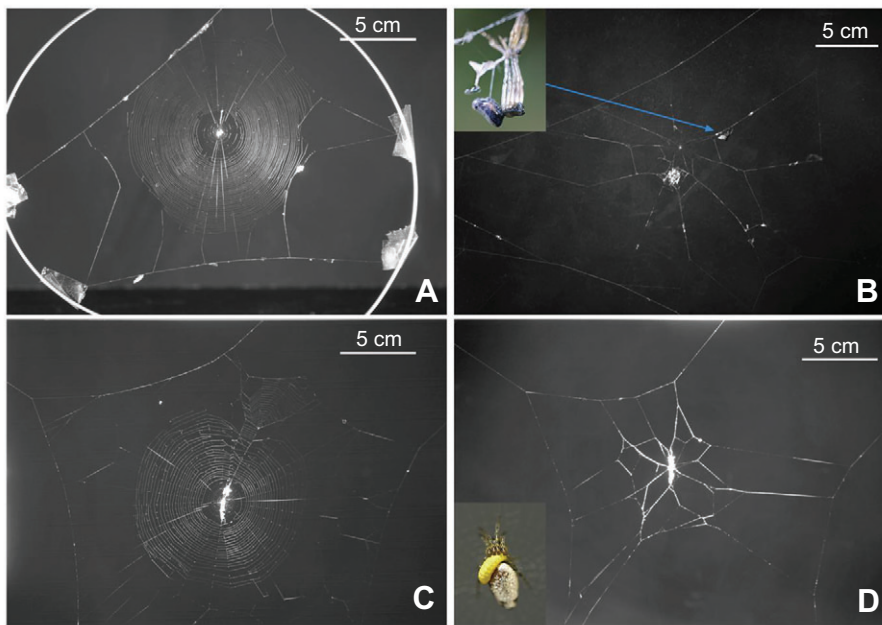


Fig. 1. Types of web constructed by the orb-weaving spider *Cyclosa argenteoalba*. (A) Normal orb web. (B) Resting web with a moulting spider (inset) somewhat away from the hub. (C) Normal orb web constructed by a spider parasitized by the ectoparasitoid *Reclinervellus nielseni* on the eve of being manipulated by the wasp larva. (D) Cocoon web constructed by the same spider as in C. Inset shows close-up of the parasitized spider.

2013; Zschokke and Bolzern, 2007); however, resting webs of *C. argenteoalba* have conspicuous fibrous thread decorations (FTDs) on the radii. The cocoon web built by a parasitized *C. argenteoalba* (Fig. 1D) is apparently similar in form to this resting web and bears FTD-like structures on the radii.

Specialized resting or ‘moulting’ webs that do not contain sticky silk and do not act as prey-capture devices are built for protection during the spiders’ vulnerable moulting stages (Blackledge et al., 2011). If a close relationship exists between a resting web and cocoon web, manipulated host behavioural processes will offer a clue to the web manipulation mechanism. Although there are several examples suggesting that parasitoid larvae trigger resting web construction (Eberhard, 2010b, 2013; Gonzaga et al., 2010), it is unclear whether the parasitoid larvae actually utilize the resting web-building behaviour, or if its resemblance is just a false impression from a shared simplicity of web structure. Solid evidence remains to be discovered.

In this study, we hypothesized that *R. nielseni* evokes resting web construction behaviour intrinsic to *C. argenteoalba* and that resting and cocoon webs are more stable than normal orb webs because both the moulting spider and wasp pupa require web durability. To test these hypotheses, we observed spider web-building behaviour and both resting and cocoon web structure in order to clarify the origin of cocoon webs. We also conducted spectral examinations of FTDs to investigate their function and determine why spiders invest time and energy in making them. Additionally, we used tensile tests to examine the mechanical property of silks that were used to build normal orb, resting and cocoon webs. If the wasp larva further manipulates resting web construction, mechanical data on silk tensile strength may reveal whether the high durability of cocoon webs is due to the modification of silk-laying behaviour, a change in silk mechanical properties (spinning stronger threads), or both. As web performance is determined by both the arrangement pattern and the mechanical properties of silk (Blackledge et al., 2011), a reinforced cocoon web must also be constructed in an advantageous way.

RESULTS

Resemblance of resting and cocoon webs

Image analysis revealed no significant difference ($t=1.5284$, $P=0.1384$) between the number of radii on resting (mean \pm s.e.,

10.95 ± 0.70 , $N=20$) and cocoon webs (9.13 ± 0.69 , $N=8$). All 20 resting webs and eight complete and three incomplete (see next section) cocoon webs had FTDs on radii. Only one complete cocoon web did not have FTDs. Meanwhile, neither resting nor cocoon webs had stabilimenta. Although superficially FTDs and stabilimenta appear similar, the fluffy structure of FTDs can be discriminated with the naked eye from the stabilimentum, which is characterized by a mass of tangled silk (supplementary material Fig. S1D) that does not wave in the wind. One of the spiders on a resting web in the field moulted, shedding its skin on a radius somewhat away from the hub (Fig. 1B inset). SEM images show that FTDs on radii of resting (Fig. 2A) and cocoon (Fig. 2B) webs are very similar in structure, but radii as a central axis of FTDs are laid down in greater numbers in the cocoon web than in the resting web.

Web-building behaviour of unparasitized and manipulated spiders

Although the whole sequence of web-building behaviour of a complete resting web as in Fig. 1B was not recorded, several specific examples of radii-decorating behaviour were seen in three individuals of unparasitized *C. argenteoalba*. All three spiders repeatedly flapped their fourth legs to spray the fibrous threads onto radii (see supplementary material Movie 1). This spraying behaviour occurred at various times depending on individuals (04:08 h, 14:00 h and 23:06 h). All spraying behaviour decorated the radii on the way back to the hub from the radial trip. One of three individuals moulted after thread decoration but shed its skin not on the hub but on one of the radii.

Recorded video revealed specific manipulated spider behaviour associated with cocoon web construction. The sequence of behavioural manipulation was stereotypical in all parasitized spiders. Manipulated spiders constructed cocoon webs on the site of their original orb web, except for one spider, which abandoned the original web and constructed a new cocoon web from scratch elsewhere, including frame thread foundation. Behavioural manipulation began with the spider retrieving the sticky spiral and then making repeated complex radial trips away from the hub and back whilst laying additional radii in both

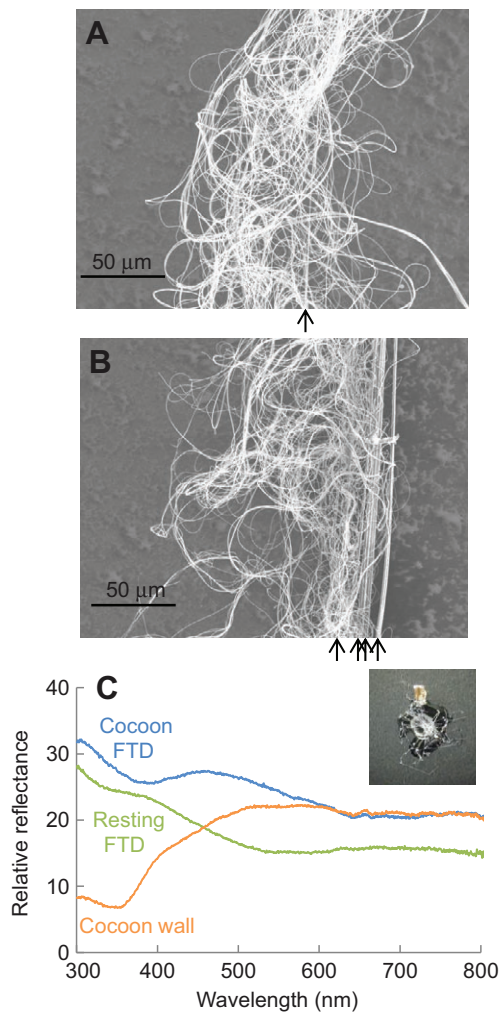


Fig. 2. Properties of fibrous thread decorations in resting and cocoon webs. Images of (A) resting and (B) cocoon webs taken with a JSM-6010LV SEM with arrows indicating various numbers of radii as a central axis. (C) Spectral properties of FTDs in resting and cocoon webs and of a cocoon wall with a piled thread sample on a hole in a polyfoam plate (see inset).

directions, without any laid threads being broken or reeled up. Retrieved sticky spirals were thrown away by waving a first leg, but were never eaten. Shuttling on frame threads for reinforcement occurred during the same trip, resulting in multiple attachment points to the substrate. At the latter phase of manipulation, the spider repeatedly flapped its fourth legs to spray fibrous threads onto radii (see supplementary material Movie 3), as unparasitized spiders do when constructing a resting web, as described above. Although FTD building frequency varied with individuals, it was concentrated in the second half of the behavioural manipulation period, lasting until the evening of the spider's final resting on the hub (supplementary material Table S1). FTDs were laid exclusively on radii, never on the frame. All the spraying behaviour occurred on the way back to the hub from the radial trip. Even if the spider decorated the radii on the radial trips away from the hub, it quickly reversed its steps and kept decorating toward the hub. After every webbing bout, the spider always sat on the hub. When cocoon web construction was finished, all spiders sat on the hub, remaining stationary until death brought about by the wasp larva. Three parasitized spiders modified only part of the orb web with FTDs (incomplete cocoon web) and one did not

modify it at all (no manipulation occurred), but in both instances the parasitoid larva killed the spider while on the hub, as in other cases.

Spiders took over 10 h on average (mean \pm s.e., 10 h 18 min \pm 2 h 24 min, $N=7$ including two incomplete cocoon webs) spinning these cocoon webs, with this phase of behavioural manipulation recognized as spanning the time taken from the withdrawal of the spiral web to the final resting of the spider on the hub of the web prior to being killed. These cocoon webs were usually constructed in darkness, between sunset and sunrise ($N=7$); however, two spiders started in the early morning or evening. The entire sequence of cocoon web construction is shown in supplementary material Movie 3. Adult wasp emergence was 10.70 ± 0.26 days (mean \pm s.e., $N=10$) following the spider host's death, under natural dark:light conditions (13.5 h:10.5 h to 14.5 h:9.5 h) and room temperature (23–27°C).

Spectral properties of FTDs and cocoon wall

FTDs in both resting and cocoon webs exclusively reflected ultraviolet light (<400 nm) prominently with uniform reflectance between 400 nm and 700 nm (visible light), whereas the cocoon wall reflected ultraviolet light less than visible light (Fig. 2C). An ultraviolet photograph of normal orb and cocoon webs shows clear white-shining FTDs in the cocoon web, demonstrating ultraviolet reflectance (supplementary material Fig. S2B). The cocoon wall was also visible, perhaps owing to its broad surface (as is the wire hoop) because UV reflectance was relatively low (Fig. 2C). The prey-capture region (sticky spiral and radii) of the normal orb web was invisible under UV light, although it was visible in a picture taken by a normal digital camera (supplementary material Fig. S2A).

Mechanical properties of thread (tensile strength)

The breaking force (mN) of both radial and frame threads was significantly greater in the cocoon web than in the other two web types, whereas there was no significant difference between normal orb and resting webs in either radial or frame threads (Fig. 3A). A close correlation was observed between thread strength and diameter (Fig. 3A,B) and no significant difference was observed in breaking stress (MPa) of each thread type (Fig. 3C), suggesting that all threads consist of materials with essentially the same mechanical properties.

The breaking strain of radial and frame threads was significantly greater in the cocoon web than in the other two web types, whereas no significant difference was observed between normal orb and resting webs (Fig. 3D) in either radial or frame threads. This suggests that some of the threads inside an accumulated thread (cocoon web) were not tensioned at the beginning of the testing, meaning that as more threads accumulate, more loose threads increase inside the accumulation. A significant difference was observed in Young's modulus of frame threads between orb and cocoon webs (Fig. 3E). In the cocoon web, some loose threads were not fixed down and tensioned as described above so did not contribute towards the breaking force measurement until these were tensioned. This resulted in an underestimate of Young's modulus, which is derived by the initial slope of the stress–strain curve, with the stress calculated by normalizing the force with the total cross section area of the thread, because accumulated silk containing loose threads produces low stress as a result of underestimated breaking force at the early stage of measurement. Indeed, there also appeared to be a distinct difference in Young's modulus of radial threads between orb/resting webs and cocoon webs, though this was not statistically significant (Fig. 3E).

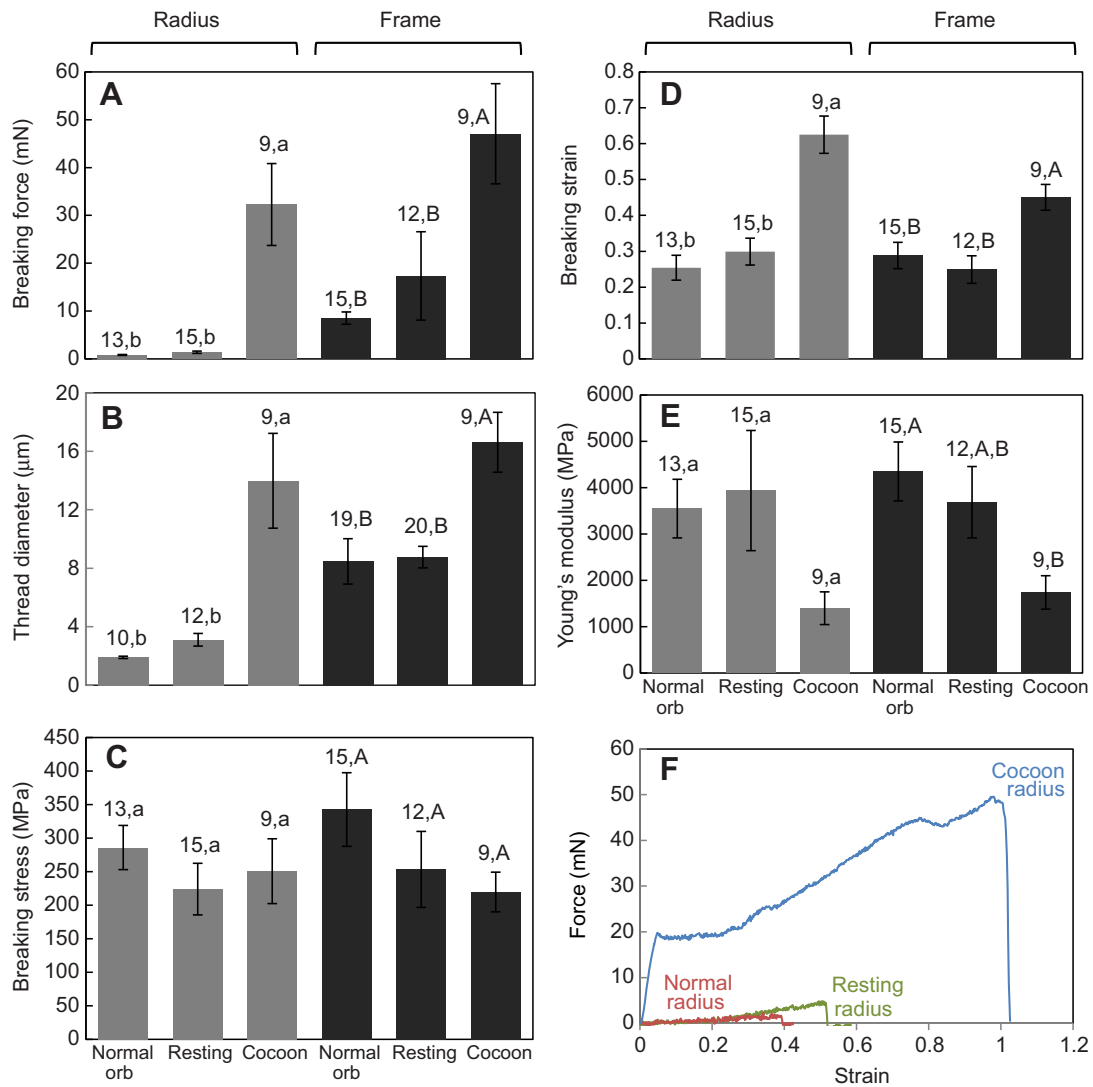


Fig. 3. Mechanical properties of radial and frame threads in normal orb, resting and cocoon webs, and typical examples of force–strain curves. Several datasets were log transformed (LT) before statistical analysis. (A) Breaking force [radius (LT): $F_{2,34}=110.728$, $P<0.0001$; frame (LT): $F_{2,33}=12.031$, $P=0.0001$]. (B) Thread diameter [radius (LT): $F_{2,28}=55.777$, $P<0.0001$; frame (LT): $F_{2,45}=8.822$, $P=0.0006$]. (C) Breaking stress (radius: $F_{2,34}=0.703$, $P=0.5023$; frame: $F_{2,33}=0.672$, $P=0.5174$). (D) Breaking strain (radius: $F_{2,34}=21.868$, $P<0.0001$; frame: $F_{2,33}=6.605$, $P=0.0039$). (E) Young's modulus (radius: $F_{2,34}=1.559$, $P=0.2250$; frame: $F_{2,33}=3.742$, $P=0.0343$). (F) Typical examples of force–strain curves of radii in three kinds of web. All bars represent the s.e. of means and figures above each bar represent number of replicates (N). Different letters (small letter in radius and capital letter in frame) denote significant differences at the 5% level of significance analyzed by the Tukey–Kramer test.

Typical examples of force–strain curves of radii in the three web types are shown in Fig. 3F. The thread strength of the cocoon web is much higher than that of the two other webs. In addition, the curve of the cocoon web shows several types of slopes, indicating that some threads inside the accumulated threads were tensioned or broken at different moments during the tensile strength test.

DISCUSSION

Origin and function of the cocoon web of *R. nielsenii*

This study demonstrates that *R. nielsenii* larvae evoke the resting web-building behaviour already programmed in *C. argenteoalba* to build cocoon webs. The radii of cocoon webs were covered with mats of FTDs, as previously seen exclusively in resting webs, with the structure (Fig. 2A,B) and building behaviour (<http://www.momo-p.com/showdetail-e.php?movieid=momo150331ca02b&embed=on>) of cocoon webs very similar to that of resting webs. Furthermore, the numbers of radii in resting and cocoon webs were reduced to the

same levels. This remarkable similarity between resting and cocoon webs supports our hypothesis.

Generally, in arthropods, feeding stops before moulting and a new set of premetamorphic behaviour triggered by ecdysteroids appears (Truman, 1992). Orb-weaving spiders also build reduced webs resulted from moulting hormone activity before moulting. The eventual webs have less area and no sticky spiral, decreasing the chance of flying insects bumping into the web and they have been described across several families of orb-weavers, such as Araneidae including *Cyclosa* (Blackledge et al., 2011; Zschokke and Bolzern, 2007), Nephilidae (Blackledge et al., 2011) and Tetragnathidae (Eberhard, 2013). Although stabilimenta in resting webs are augmented before moulting in some species (Blackledge et al., 2011; Robinson and Robinson, 1973; Walter et al., 2008), only one species, *Gasteracantha cancriformis* (Araneidae), had previously been known to build resting webs with FTDs (stabilimentum tuft) randomly on radii inside the orb (Eberhard, 2007; Jaffé et al., 2006).

Therefore, *C. argenteoalba* is the second recorded species to exhibit FTDs in resting webs, although these differ in decoration length from those of *G. cancriformis*.

The fact that two unparasitized *C. argenteoalba* each moulted on a resting web (Fig. 1B) or decorated thread indicates a close relationship between the moult of spiders and the use of a resting web with FTDs. As resting webs lack the sticky spiral, the possibility that the web is used for predation can be entirely dismissed. The FTDs of *C. argenteoalba* reflects mostly UV light in both resting and cocoon webs (Fig. 2C), probably making the web conspicuous to potential web-destroyers such as birds and large flying insects. Tufts decorating *G. cancriformis* webs also had a flat spectrum with a 15% reflectance, including the UV component (Gawryszewski and Motta, 2008) and tufts at least on resting webs were thought to serve as advertisements (Eberhard, 2007; Jaffé et al., 2006). The presence of FTDs thus agrees with the web-destroyer deterrence hypothesis, by advertising the presence of the web that is home to both the fragile moulting spider and the non-feeding wasp pupa. *Reclinervellus nielseni* larvae apparently utilize the host spider's behaviour in which it builds webs primarily for moulting and expresses this behaviour in a different context, i.e. building a cocoon web; this behavioural manipulation takes advantage of the original web function, of being conspicuous and ineffectual for catching prey, to protect the wasp pupa in the cocoon web.

Interestingly, the tensile strength (breaking force) and diameter of both radial and frame threads in the cocoon web were significantly greater than those in normal orb and resting webs (Fig. 3A,B), proving that cocoon webs are more durable than resting webs. However, the same level of breaking stresses of both radial and frame threads amongst the three types of web (Fig. 3C) suggest that *R. nielseni* does not manipulate any properties of silk produced by the host spiders. The wasp larva thus evokes the resting web-building behaviour and induces repetition of the behavioural subset of radial and frame weavings. A relatively long time is needed for this behavioural manipulation (over 10 h on average) and repeated radial trips and frame shuttling as shown in supplementary material Movie 3 support the idea that certain behavioural steps from the construction of resting webs are repeated many times to create a cocoon web. The accumulated threads in the cocoon web (supplementary material Fig. S1A,B) are also evidence of repeated weavings because the twin spigots of the major ampullate gland in most spiders, which spin radial and frame threads (Coddington, 1989; Yoshida, 1999), always produce a single thread consisting of paired fibres (Blackledge et al., 2011). A piece of thread from either accumulated radial or frame threads in the cocoon web (supplementary material Fig. S1A,B) has the same diameter (about 1–2 µm) as the radius of a normal orb web, which consists of a pair of threads, with each being also about 1–2 µm in diameter (supplementary material Fig. S1C).

The tensile strength and diameter of both radial and frame threads also show no significant differences between normal orb and resting webs (Fig. 3A,B). This suggests that the resting web is designed to be conspicuous but is not reinforced. Spiders in their moulting phase (presumably a couple of days) may require less persistence of the resting web, whereas the wasp larva demands a more persistent cocoon web to persist through its pupal period of over 10 days. The pupa can be exposed to flying creatures inadvertently crashing into the web and falling debris much more frequently, driving this *ad hoc* manipulation procedure (evocation of resting webs and repetition of thread weavings).

Several recent studies have suggested that polysphinctine larvae manipulate host spiders to make a tougher structure for more

persistent cocoon webs, to enhance parasitoid pupae survival (Eberhard, 2000, 2001, 2010b, 2013; Gonzaga and Sobczak, 2011; Gonzaga et al., 2010; Korenko et al., 2014; Matsumoto and Konishi, 2007; Sobczak et al., 2009). However, to our knowledge, the present study is the first to show the durability of cocoon webs compared with normal webs with regards to their mechanical properties.

Web decorations in orb web spiders

Although web decorations, including stabilimenta, are described exclusively in diurnal orb web spiders (Blackledge et al., 2011), their function has been controversial, with several hypotheses proposed, probably because decorations have different functions depending upon species (Herberstein et al., 2000). Two hypotheses proposing a visual signalling function – anti-predator (including camouflage from predators and advertisement towards web-destroyers) and prey attraction – have been predominantly studied (Bruce, 2006). However, these two hypotheses conflict because spiders simultaneously need to avoid predators and attract prey, making experimental design to exclude either possibility complicated (Blackledge, 1998; Bruce et al., 2001). In the most intensively studied group, the genus *Argiope*, the hypothesis that its ribbon-like zigzag stabilimenta function to deter bird strikes by means of web advertisement is generally supported (Blackledge and Wenzel, 1999; Eisner and Nowicki, 1983) but the evidence is not yet conclusive.

Linear stabilimenta in webs constructed by *C. argenteoalba* increase both in number (not significantly) and length (significantly) after webs are exposed to simulated predatory insect flutter, by means of a tuning fork, but this increase is not related to prey availability, suggesting that the stabilimenta have a predator defence function (Nakata, 2009). However, it is unclear whether this acts as camouflage or advertisement, or what the target predator is. Although the functions of the linear stabilimenta and FTDs generated by *C. argenteoalba* are similar in terms of deterring predators, ultimate mechanisms would be disparate because their forms, circumstance of their appearance, locations in the web and building costs are distinct; it is possible, for example, that the stabilimenta camouflage the spider from specific predators (not yet verified), whereas FTDs make the web conspicuous to any flying animals.

Stepping stone to elucidation of proximate mechanisms of web manipulation

Elucidating the proximate mechanism of host manipulation is very complicated (Thomas et al., 2005) and remains unsolved for polysphinctines. Eberhard (2010b) suggests that larval effects may depend on a cumulative or dose-dependent process rather than multiple products each responsible for influencing independent behaviours, on the basis that removing parasitoid larvae caused the spiders to recover their normal web-building behaviour in the reverse order from when they had been building cocoon webs. Where polysphinctine larvae evoke a clearly innate web construction, which appears exclusively in a specific life-history stage, it is suggested that the wasp larvae produce a signal molecule responsible for the onset of such behaviour (Korenko and Pekár, 2011). The *ad hoc* manipulation in our study system provides additional evidence.

One striking finding in the present study is that *R. nielseni* larvae force the host spiders into repeatedly weaving the threads, which perhaps occur less in resting web construction, retaining other building steps (retrieving sticky spiral webs and FTD construction) as normal. A behavioural sequence of resting web construction can thus be subdivided into several independent subsets. *Reclinervellus nielseni* is able to control the recurrence of a particular subset

independently, as in another polysphinctine, *Hymenoepimecis argyraphaga*, which forces a tetragnathid orb-weaver into repeating the early steps of one subset of normal orb construction, repressing other behavioural patterns (Eberhard, 2000, 2001).

As resting web construction would be governed by ecdysteroids, it is possible that *R. nielsenii* injects chemical components corresponding to moulting hormone into host spiders, causing the onset of resting web construction, supporting the signal molecule hypothesis. However, *R. nielsenii* has to manipulate *C. argenteoalba* not to moult. As the ecdysteroidal concentration gradient causes several behavioural responses in juvenile insects in a dose-dependent manner, as seen in tobacco hornworm (Truman, 1984), resting web construction consisting of several sequential building behaviours is likely to result from the same effect, and the manipulative substance may react with the spider's endocrine system similarly, supporting the dose-dependent hypothesis. The repeated thread weavings absent in resting web construction may be brought about by an unusual concentration of such a manipulative substance. Physiological and biochemical approaches to investigate this further are needed in future studies.

MATERIALS AND METHODS

Field survey and rearing methods

Thirty-six *R. nielsenii* larvae on their host spiders, *C. argenteoalba*, were collected in Tamba and Sasayama cities, Hyogo Prefecture, Japan, in the months of April and May from 2012 to 2014. The parasitism rate was relatively low. Five day searches in spring (April to May) in 2014 produced 23 larvae from a survey of 1615 host spiders (1.4%), whilst 4 day searches in autumn (October to November) in 2013 produced no larvae from a survey of 638 host spiders. In the study sites, we inspected spiders caught from orb webs, tracking in a designated way so that no spider was counted twice. The spiders were individually placed in an empty vial and inspected with a loupe for parasitism, and then unparasitized spiders were returned to the original web. All parasitized spiders were juveniles. Eleven of the parasitoid larvae reached the pupal stage, providing us with 10 cocoon webs (three incomplete). Other individuals did not complete development because the host spiders died before the wasps completed feeding. Several unparasitized juvenile spiders were also collected to observe their behaviour when constructing resting webs. We took pictures of resting webs in the field and cocoon webs from laboratory rearings (and one in the field) to count the number of radii in resting and cocoon webs radiating from the central disc, considering multiple radii converging upon the outer part of the web to be one radius (supplementary material Fig. S3). In total, 154 orb webs and 19 resting webs (about 11%) of unparasitized spiders were found in one day's field investigation. Incomplete cocoon webs were not included in the radii counting survey.

To remove spiders and their webs from the field, we used a rounded wire hoop held on a wooden stand for sustaining the webs by fastening the anchor lines of the web to the hoop with cellophane adhesive tape as shown in Fig. 1A. If the spider captured in the hoop retrieved its web and left, we placed the spider in a transparent acrylic frame (45×45×6 cm) covered with two transparent acrylic boards (50×50×0.5 cm) to encourage it to rebuild the web. The wire hoops and the acrylic frames with spiders were maintained in the laboratory under natural dark:light conditions and room temperature (23–27°C). Spiders were fed with flies (mainly chironomids) every 2–3 days until the wasp larva completed its growth. Voucher specimens will be deposited in Osaka Museum of Natural History, Osaka, Japan.

Web characteristics of *C. argenteoalba*

Unparasitized *C. argenteoalba* constructs a vertical orb web (Fig. 1A) with a prey-capture region that is fine and delicate and occasionally overlooked by the naked human eye. The spider always sits on the hub of the web awaiting prey and builds neither a barrier web beside the orb web, as is frequently observed in *Nephila*, *Araneus* and *Argiope*, nor a retreat to hide in. There are two types of decorations found in a web: FTDs and vertical hub decorations (stabilimenta). FTDs are fluff-like structures laid randomly along the reduced numbers of radii in a resting web, in the area that functions as a

'capture region' in a normal orb web. The stabilimentum comprises one or two almost vertically aligned linear silk decorations above and below the hub of the normal orb web where the spider sits and it forms a mass of tangled silk on several radii (supplementary material Fig. S1D) that do not wave in the wind. Normal orb webs without stabilimenta are also frequently found (Nakata, 2009).

Observations on cocoon webs and resting webs, including web-building behaviour

We used a digital single-lens camera, DMC-GH1 (Panasonic Corporation) with a macro lens (Leica DG Macro-Elmarit 45 mm/F2.8 Asph.) to record high-definition videos of unparasitized spider web-building behaviour during resting web construction and manipulated spider web-building behaviour during cocoon web construction. We could anticipate the initiation of cocoon web construction by the size of the wasp larva. When manipulation (cocoon web construction) occurred through the night, we illuminated the web with red LED light so as not to influence the photoperiod of either the spider or the wasp larva. However, it is impossible to anticipate when a spider will begin to build a resting web, so we blindly recorded videos of the spider sitting on the hub of the normal orb web. Videos were recorded in monochrome with maximum exposure to capture the fine threads.

Spectral properties of FTDs and cocoon wall

The fibrous decorated radii in resting and cocoon webs were accumulatively piled in the flat over a 5-mm-diameter hole perforated in a polyfoam plate and surrounded by an adhesion bond (Ultra tayouto SU, Konishi Co. Ltd) (Fig. 2C inset). The relative reflectance of piled thread samples and a wasp cocoon were measured for wavelengths between 300 and 800 nm. We used a USB 2000 spectrometer (Ocean Optics Co.) connected to a DT-MINI-2-GS light source (Ocean Optics) and a WS-1 diffuse reflectance standard. The reflectance data were obtained with a PC running OOIBase32 software.

Mechanical properties of threads (tensile test)

The radial and frame threads of three kinds of web (normal orb, resting and cocoon web) all built by medium-sized juvenile spiders were tested by a tensile machine (AGS-J, Shimadzu Corp.) to obtain breaking force (mN), breaking stress (MPa), breaking strain and elastic modulus (Young's modulus; MPa). Nine cocoon webs including two incomplete cocoon webs (see also Results) were utilized for the test. All mechanical tests were performed in the air at room temperature and replicates for each category are shown in Fig. 3. The threads were extracted and fastened by cellophane adhesive tape onto cardboard cut in a horseshoe-shape with a regular interspace of 10 mm (initial length). When testing the incomplete cocoon webs, we extracted only the modified part of the cocoon webs that were visually recognizable or observed on the video data. Each silk cardboard was vertically fixed with two pairs of tweezers and then the cardboard was cut medially to split it into two pieces so that the tester pulled only on the silk sample. After setting in the machine, it was extended at a constant elongation rate of 10 mm min⁻¹ until the silk sample was broken. We repeated the tensile test multiple times for almost all web samples (excluding frame threads of normal orb and resting webs) and averaged results for each web sample. The breaking stress was obtained by dividing the breaking force by the cross-section area of the thread. To calculate thread cross-section areas, we took micrographs of the threads on glass slides using a biological microscope (Axioskop 2 plus, Carl Zeiss Microscopy Co. Ltd) at 400× magnification before the tensile test and measured thread diameter with the open source software ImageJ with a micrometer. We determined the representative diameter of each thread by averaging ten measurements of diameter using image analysis. Young's modulus was estimated from the initial slope of the stress-strain curve. All data obtained through the tensile test with force-strain curves are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.3bf67>.

Statistical analysis

Five datasets (breaking force, thread diameter, breaking stress, breaking strain and Young's modulus) divided into two categories (radial and frame

threads) were each analyzed by one-way analysis of variance (ANOVA) with respect to web types (normal orb, resting and cocoon web) and then analyzed with the Tukey–Kramer test for multiple comparisons as a *post hoc* test using the package R (R Developmental Core Team, 2013). Before analysis, breaking force values and thread diameter were transformed into natural logarithms, bringing variance within allowable differences amongst groups. Statistical significance was set at $P < 0.05$ for all experiments.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

K.T. designed the experiments, collected field samples and wrote the manuscript; T.Y., T.I. and K.T. performed the tensile test and analyzed obtained data; K.N. and K.T. performed wavelength measurements; K.I. and K.T. took the electron micrographs; K.M. and K.T. participated in statistical analysis; R.M., K.N. and K.M. helped draft the manuscript.

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Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.122739/-DC1>

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