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# Oviposition behaviour by a spider-ectoparasitoid, *Zatypota maculata*, exploits the specialised prey capture technique of its spider host

Short title: Oviposition behaviour of *Zatypota maculata*

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# Abstract

Oviposition on or into a living animal is an arduous undertaking for parasitoids, including in terms of counterattack or evasion by the host. Parasitoids of active spiders protected by their own webs (polysphinctine spider-ectoparasitoids in the family Ichneumonidae) need to avoid entanglement with the spider's web to gain access to the spider host for oviposition. These circumstances have driven the evolution of offensive behaviours highly adapted to the web architecture of each spider host. We discovered offensive oviposition behaviour in a member of the *Polysphincta*-group, *Zatypota maculata*, parasitising a theridiid spider, *Nihonhimea japonica*, which constructs a "knockdown 3D web". It consists of a non-viscid 3D structure above, with a retreat (a dead leaf) hung at the centre, and a non-viscid sheet below as a capturing device. When a prey insect is knocked down and then trapped by the sheet, the spider immediately drops onto the underside of the sheet by penetrating the sheet to bite and wrap it. *Zatypota maculata* exhibits two behaviours to deal with this type of web: 1) the wasp climbs the 3D cobweb, creeps up slowly onto the spider's retreat, taking a long time so that the spider does not escape, and finally enters the retreat to sting the spider (creeping-style); 2) the wasp dives from the outside of the web onto the sheet, as a knocked-down prey item would, to lure the spider out, before stinging it at the moment of contact (diving-style). We discuss these unique offensive behaviours and compare them with behaviours of other polysphinctines and spider-hunting aculeates. Ovicide by means of removal of pre-existing eggs was also observed under experimental conditions.

**Keywords**: aggressive mimicry; behavioural plasticity; ectoparasitoid; ovicide; oviposition behaviour; polysphinctine; knockdown 3D web, Theridiidae, Ichneumonidae.

# Introduction

Oviposition on an active host obliges the parasitoid to face risks of counter-attack or evasion by potential hosts (Greeny *et al.* 2012, Gross 1993), which at worst cause the parasitoid's death. For example, a braconid endoparasitoid of ants, *Elasmosoma luxemburgense* Wasmann 1909, was witnessed being caught by a host ant worker (Gómez Durán & van Achterberg 2011). These risks never happen in oviposition by phytophagous, saprophagous, and even predatory arthropods because their oviposition is never on living animals, suggesting that the parasitoids' oviposition requires specific and offensive oviposition behaviour to breach protective behaviours of host taxa.

Among hymenopteran parasitoids, there is a group parasitising active predatory spiders called the *Polysphincta* group of genera (Hymenoptera, Ichneumonidae; hereafter polysphinctine). This is a monophyletic lineage ecologically characterised by being solitary koinobiont ectoparasitoids of spiders with high host specificity (Gauld & Dubois 2006, Matsumoto 2016) and currently comprises 25 genera and over 250 species distributed all over the world (Matsumoto 2016, Yu *et al.* 2016). To oviposit upon spider host protected by their own webs, polysphinctines must have evolved ways of offensive (i.e. approaching and attacking) oviposition behaviour that is highly adapted to the web architecture of each spider host.

In fact, the offensive oviposition behaviours of polysphinctines have been documented in several species, which exhibited highly specific strategies to deal with the web architecture of each spider host (Eberhard 2000, Gonzaga & Sobczak 2007, Iwata 1942, Kloss *et al.* 2016, Matsumoto 2009, Takasuka & Matsumoto 2011b, Takasuka *et al.* 2009, Weng & Barrantes 2007). Such behavioural records provide us with invaluable clues in discussing host shifts and speciation history of polysphinctines because oviposition is the initial event of new host utilisation. However, data are still very scarce because of the rarity of witnessing these behavioural events *in situ* and the difficulty in evoking them *in vitro*, despite there being over 250 described polysphinctine species.

The polysphinctine genus *Zatypota* is the largest of the genus-group, with 50 described species (Yu *et al.* 2016), with members in general parasitising spiders of the family Theridiidae (cf. Matsumoto & Takasuka 2010) which typically construct three-dimensional cobwebs that exclude ‘sneaking’ predators of spiders and kleptoparasites (spiders that steal prey or usurp webs) (Murphy & Roberts 2015) (a minority of *Zatypota* species are parasitoids of the families Araneidae, Linyphiidae, and Dictynidae; see Aubert 1969, Fitton *et al.* 1988, Gauld & Dubois 2006, Korenko 2017, Korenko *et al.* 2015, Matsumoto & Takasuka 2010, Vincent 1979). Spiders of the family Theridiidae are thought to successfully avoid predation by spider-hunting wasps of the families Sphecidae and Crabronidae by means of their 3D web architectures, based on the low frequency of predation records of theridiids in comparison with that of 2D orb weaving spiders (Blackledge *et al.* 2003), excluding *Chalybion* (Hymenoptera: Sphecidae) species, which exceptionally prefer theridiids, in addition to other spiders (e.g. Landes *et al.* 1987, Muma & Jeffers 1945, Ward 1969). The adoption of theridiids as hosts by *Zatypota* spp. has involved the successful evolution of specific offensive behaviours to overcome the intricate defensive 3D web architectures.

*Zatypota maculata* Matsumoto & Takasuka 2010, on which we focus, exclusively parasitises the theridiid spider, *Nihonhimea japonica* (Bösenberg & Strand 1906) (Matsumoto & Takasuka 2010), which was recently transferred from *Parasteatoda* by Yoshida (2016). All three species of the genus *Nihonhimea* construct a characteristic web called a “knockdown 3D web”, which consists of a non-viscid intricate 3D cobweb, a retreat made of a dead leaf at the centre, and a dense non-viscid sheet web at the bottom that serves as a capturing device (Figs 1, 4c, Benjamin & Zschokke 2003; Fig. 6C, Eberhard 1972, Eberhard *et al.* 2008; Fig. 42D-F, Jörger & Eberhard 2006; Figs 9, 19, Yoshida 2003, Yoshida 2016; Figs 18-19). At the moment a prey insect is knocked down by the cobweb and trapped by the sheet, the spider immediately drops down from the retreat onto the underside of the sheet by penetrating the sheet, resulting in a small hole on the sheet. It them bites and wraps the captured prey from underneath (cf. Takasuka 2013).

The knockdown 3D web seems to be particularly effective in avoiding predation by spider-hunting aculeates because *N. japonica* has been scarcely recorded as stored prey of sphecid (Sceliphrini), crabronid (Trypoxylini), or pompilid wasps. There is only a record of *N. japonica* stored by *Pison* (*Pison*) *punctifrons* Shuckard, 1838 (Crabronidae), but just one individual among 74 individuals from four families, Theridiidae (mainly *Parasteatoda tepidariorum* (C. L. Koch, 1841)), Araneidae, Tetragnathidae, and Salticidae (Iwata 1964). However, *Z. maculata* has successfully evolved offensive oviposition behaviours specifically to cope with the knockdown 3D web of *N. japonica*. In the present study, we report observations on these unique offensive oviposition behaviours of *Z. maculata* both *in situ* and *in vitro*, with the establishment of an observational system *in vitro*.

## Materials and methods

### Observation of oviposition behaviour *in situ*

We conducted field observations in two periods and locations in Japan, Satsukiyama, Ikeda city, Osaka Prefecture, in 2006 by RM, and the campus of Kobe University, Kobe

City, Hyogo Prefecture in 2015 by KT; however, there were no observations on oviposition success at the latter. Instead, the campus played a role in being a resource of spiders and adult wasps for the oviposition experiments *in vitro*. The observed spiders constructed their webs on planted *Osmanthus fragrans* Lour. var. *aurantiacus* Makino (Oleaceae) in Ikeda and on *O. fortunei* Carrière (Fig. 1a) in the Kobe University campus. A survey of the spider host population on 4 September 2015 on the campus resulted in 49 parasitised spiders (44 eggs, four young and one old instar larvae) out of 62 individuals, and four cocoons inside empty retreats, resulting in 80.3% (53/66) parasitism. Almost all spiders bore egg sac(s) or hatched spiderlings inside their retreats. Sequences of female wasps' behaviour in Ikeda were recorded using a digital single lens reflex camera, Nikon D100 (Nikon Corporation), by RM.

### Observation of oviposition behaviour *in vitro*

Several *N. japonica* were collected together with their own retreat (dead leaf) and incorporated into an artificial substructure for web construction. It consisted of two corrugated fibreboards cut in a doughnut-shape above and below, connected by wooden sticks (Fig. 1b). We hung the substructure with a knockdown 3D web and its owner inside a large plastic reservoir (60 cm × 43 cm × 30 cm) laid sideways. After closing the reservoir with a fine mesh cloth, three adult female *Z. maculata* wasps (individuals A, B and C) collected on the campus were introduced into the reservoir one at a time. They were fed with a sugary isotonic drink (Aquarius, manufactured by Coca-Cola Japan), which achieved maintenance of another polysphinctine (*Z. albicoxa*) for a long time (Takasuka & Matsumoto 2011b, Takasuka *et al.* 2009). The wasp was replaced with another individual when she died, and spiders that had not been attacked were re-used in the succeeding experiment. We recorded the wasps' oviposition behaviour with a digital single lens camera DMC-GH1 (Panasonic Corporation) fixed on a tripod. We judged an attack as successful by the spider's paralysis caused by the wasp's sting.

In addition to normal oviposition, we qualitatively tested infanticidal behaviour. In other polysphinctines, it is known that previously attached immature wasps (previous occupants) on a spider host are removed by a subsequent ovipositing female wasp with rubbing movements of her ovipositor (Eberhard 2000, Gonzaga & Sobczak 2007, Takasuka & Matsumoto 2011a). We provided a parasitised spider, which remarkably bore both a middle instar larva and an egg (i.e. it was superparasitised) once each to individuals B and C, i.e. the same individual spider was used twice.

## Results

### Oviposition behaviour *in situ*

A female was witnessed for the entire sequence of oviposition. In the first phase, the wasp stayed on a leaf below the sheet, to which one of the supporting structures was attached. The wasp flew up to cling to one of the upper outermost supporting structures (Fig. 2a) and crept around along it. Then, she flew again, landed on the lower supporting structure and slowly climbed up and down threads of the supporting structure and knock-down trap (sheet), towards the retreat, facing the retreat (Fig. 2b). Once she reached the spider host's retreat, the spider escaped for a short distance, approximately 25 mm away from the retreat (Fig. 2c), before the wasp made contact. The wasp remained motionless with her fore legs raised beside (Fig. 2c) and approximately 5 minutes later, on the spider's return to the retreat, she pounced and quickly stung it, causing temporary paralysis. She grabbed the spider's abdomen and legs with her fore and middle legs (Fig. 2d), and gave several additional stings into the bases of the coxae. Subsequently, she repeatedly rubbed her ovipositor over the anterior and lateral faces of the host abdomen. Then, an egg was laid on the anterolateral face of the host abdomen. It took approximately 15 minutes and 30 seconds for the wasp to reach the retreat and the duration between sting and oviposition was 260 seconds. Approximately 15 minutes later, the spider recovered from paralysis and hid herself inside the retreat listlessly.

In an incomplete observation on another individual, in which the wasp was already inside the supporting structures above the sheet and rather close to the retreat, the wasp similarly crept toward the retreat and then entered it. She succeeded in paralysing and ovipositing on the host without it escaping.

### Oviposition behaviour *in vitro*

In the oviposition experiment, five instances of successful oviposition (zero by wasp A, four by B, and one by C) were observed and two types of behaviour to gain access to spiders were recognised: 1) creeping up on a retreat as observed *in situ*; 2) diving into a sheet to lure a spider away from a retreat. Oviposition was observed on 5, 8, 11, and 15 September 2015 by wasp B and on 12 September 2015 by wasp C.

The typical "creeping-style" was observed in individual B (Supplementary video 1). We released the wasp from an empty vial to the lower fibreboard of the web substructure. She jumped 1 cm upwards, lay on her dorsum on the rear of the sheet, and then pulled herself through the sheet. Walking on the sheet to an edge of it, she climbed up the 3D cobweb to the part of the web above the retreat in which the spider and its spiderlings hid and climbed up and down repeatedly for approximately 24 minutes, gradually creeping up on the retreat, with several periods of motionlessness. Whilst the wasp moved around the web, the spider stayed still in the retreat, with no observed reaction. The wasp finally stopped 1 cm away from the retreat for 230 seconds before eventually entering the retreat, resulting in the spider being stung and paralysed, and also the spillage of several spiderlings. When the spider was paralysed, she grasped the spider's abdomen by her fore and mid legs and rubbed it with her ovipositor and tip of metasoma repeatedly at short intervals. Stopping metasoma movement, she laid an egg onto the anterolateral face of the spider's abdomen. The duration between sting and oviposition was 202 seconds. After oviposition, she left the retreat, rested for 58 minutes 30 seconds on the outer side of the retreat and finally flew away from the web. The spider first moved again nine minutes after oviposition and a strange behaviour was observed. As soon as the spider had recovered movement, it suddenly bit and wrapped up one of its spiderlings and left it immediately outside the retreat. The spider re-entered the retreat without eating it.

Another three successes by wasp B were accomplished by means of the typical "diving-style" (Supplementary video 2). She directly dived into a sheet from the air, the wooden stick or the lower fibreboard of the web substructure. Immediately after the wasp dived into the web, the spider dropped or climbed down from the retreat and approached her as potential prey along the underside of the sheet. At the moment of contact, the wasp stung and paralysed the spider. In one case, the spider approached her but was very cautious, returning to the retreat twice while the wasp feigned struggling on the sheet the entire time. At the third approach of the spider to the supposed prey, she pounced upon the approaching spider from 1 cm away. In all three cases, the rubbing behaviour was observed before oviposition, as described above. The duration between sting and oviposition was 220, 225, and 245 (including ovicide) seconds, respectively.

Behavioural plasticity during a bout of oviposition by wasp C (Supplementary video 3) was observed. After she dived into the sheet from right above the web through a hole in the upper fibreboard (“diving-style”), but the spider, which obviously reacted to her diving, did not approach her. Being in a stalemate for 150 seconds, the wasp climbed up the 3D cobweb and the spider subsequently climbed down as if she was potential prey, resulting in the spider being stung. This appears to be a “fall-back method of luring” by aggressively climbing following the failure in elicitation of the desired response from the spider by diving. The rubbing behaviour was observed and the duration between sting and oviposition was 251 seconds (including ovicide). In this case, she was clearly observed to pull her metasoma inwards and expelled an egg from the proximal end of the ovipositor (Fig. 3), i.e. neither from the ovipositor tip nor the tip of the metasoma (the genital opening). This oviposition stance is called “ventral-press” *sensu* Takasuka *et al.* (2018), an adaptation to ovipositing on spiders’ abdomens and preserved well within one of two clades of polysphinctines (the other clade exhibits the reverse movement for laying on a spider’s cephalothorax). This is the fifth record of the ventral-press in this clade.

Wasp A was not observed to successfully attack a spider, but some attempts to gain access to the spider were observed. She frequently perched on the mid-height of the cobweb (supporting structures) and sometimes subsequently dropped onto and walked on the sheet. In one case, she crept up on the retreat but the spider dropped off it and stayed motionless on the periphery of the sheet. She entered the empty retreat, stayed there for four minutes, and was finally reconciled to flying away.

## Ovicide

When superparasitised spiders, bearing both a middle instar wasp larva and an egg, were provided to wasps B and C, the same results occurred. After 15-30 seconds of rubbing behaviour after the first sting, they immediately detected the egg and levered it off with the ovipositor tip, removing the egg in merely 10-20 seconds (Supplementary videos 2, 1:07-1:30; 3, 1:05-1:17). The removed egg attached to the ovipositor was eventually discarded by wiping her hind leg (Supplementary videos 2, 1:48; 3, 1:17). She focused the rubbing behaviour exclusively on the left side of the spider's abdomen where the egg was attached and made additional stings into a membrane between the left coxae (Supplementary videos 2, 2:15-2:23). She finally laid an egg onto the left side of the abdomen dorso-laterally and flew away without removing the existing larva on the right side of the abdomen, which was right in front of her. Thus, she was superparasitising the spider again. We examined one of the removed eggs under the microscope and observed embryonic movement inside the egg chorion, suggesting that the egg was not killed by the removal. Although the additional sting observed in both cases with ovicide was not detected in ovipositions upon unparasitised spiders, they were not clearly recorded in the videos due to the position of the spider and the resolution of the videos.

## Discussion

### Oviposition tactics of *Z. maculata* with comparative ethology

We discovered behaviours of *Z. maculata* that are presumably species-specific, being specialised to the knockdown 3D web of *N. japonica*. *Zatypota maculata* females clearly recognised the sheet web, interpretable from their avoidance (creeping-style) and exploitation (diving-style) of the web. This is the third observation of oviposition behaviour of polysphinctines against theridiid spiders (all by *Zatypota*) and there are both unique and mutual characteristics among ours and the other two records. The comparison is schematised in an ethogram (Fig. 4).

*Zatypota petronae* Gauld, 1991 utilises *Theridion evexum* Keyserling, 1884 found in Costa Rica (Barrantes & Weng 2007). The web of *T. evexum* includes a folded live leaf that forms a conical retreat, with a tangle in front of the retreat opening, and long viscid lines extending from the tangle to other leaves (Fig. 4a, Eberhard *et al.* 2008; Fig. 20A-B, Weng & Barrantes 2007). A female *Z. petronae* hovered in front of the retreat opening and then flew inside it, resulting in a struggle between the wasp and spider hanging on a dragline (Weng & Barrantes 2007). The spider was finally paralysed, although the authors did not confirm whether an egg was eventually laid.

*Zatypota albicoxa* (Walker, 1874) utilises *Parasteatoda tepidariorum* and several other congeners across the Palaearctic region (Bordoni 2003, Fitton *et al.* 1988, Iwata 1942, Matsumoto & Takasuka 2010, Nielsen 1923). The web of *P. tepidariorum* consists of a central retreat (generally made of silk thread tangles, but rarely of a dead leaf or detritus) and numerous gum-footed vertical lines to suspend prey in the air (Fig. 4b, Benjamin & Zschokke 2003; Figs. 6A, 8A, Griswold *et al.* 1998; Fig 2A-B, Takasuka & Matsumoto 2011b; Fig. 1). Females of *Z. albicoxa* have evolved several tactics for coping with the gum-footed cobweb to gain access to *P. tepidariorum* (Fig. 4b): 1) by perching on and hanging from one of the gum-footed lines to lure the spider (ambush-style; Takasuka 2009b, Takasuka *et al.* 2009); 2) by perching on the mid-height on the cobweb (non-sticky area) to lure the spider or to pounce directly on the spider when it is passing by (alternative ambush-style; Takasuka 2009a, Takasuka *et al.* 2009); 3) by climbing up a non-sticky frame thread from the ground to gain access to the spider directly (climbing-style; Takasuka *et al.* 2009); and 4) by wandering under the web and laying on her dorsum on the floor pulling at a gum-footed line with her fore/mid legs to lure the spider (reclining-style; Takasuka 2011a, b, c, d, Takasuka & Matsumoto 2011b).

The two types of tactics employed by *Z. maculata* observed in this study (creeping to gain access and the diving to imitate prey, Fig 4c) seem to demonstrate phenotypic plasticity within an individual because both behaviours were practised by two individuals, A and B (C showed plasticity by diving and climbing as a fall-back method). The deployment of creeping-up tactics in both *Z. maculata* and *Z. albicoxa* (Takasuka *et al.* 2009) possibly indicates that this tactic is effective and potentially widely shared in the genus *Zatypota* against theridiid 3D cobwebs, whose threads are mostly non-viscid. The imitating tactic (aggressive mimicry), adapted to various types of theridiid cobwebs would thus evolve differently in each host-parasitoid interaction. However, offensive behaviour of other members of *Zatypota* (the most species-rich polysphinctine genus) remains to be discovered.

Offensive behaviours of *Zatypota* indicate that to subjugate spiders *Zatypota* wasps adopt careful ways to approach so that the spiders do not escape from the wasp, rather than avoiding becoming prey to the spiders, by means of an abrupt plunge (*Z. petronae*), creeping-up (*Z. albicoxa* and *Z. maculata*), and aggressive mimicry, i.e. imitation of prey and ambush (*Z. albicoxa* and *Z. maculata*) (Fig. 4, Table 1). Indeed, all failures in oviposition in this study resulted from spiders escaping as a reaction to the proximity of the wasp. Stealth or luring of the spider has also been observed in other genera of polysphinctines known for their offensive oviposition behaviour (Table 1), including a plunge by *Hymenoepimecis argyraphaga* Gauld, 2000 and *H. veranii* Loffredo & Penteado-Dias, 2009, aggressive mimicry by *Brachyzapus nikkoensis* (Uchida, 1928), and pure ambush (i.e. no imitation) by *Polysphincta* sp. nr. *purcelli* Gauld, 1991 and *P. janzeni* Gauld, 1991 (Eberhard 2000, Gonzaga & Sobczak 2007, Iwata 1942, Kloss *et al.*

2016, Matsumoto 2009).

These conservative tactics among polysphinctines contrast with spider-hunting aculeates; many species of the family Pompilidae (Eberhard 1970, Preston-Mafham 1991, Shimizu 1989), *Sceliphron* (Sphecidae, Sceliphrinae) (Blackledge & Pickett 2000, Eberhard 1970), and *Miscophus* (Crabronidae, Miscophini) (Coville 1986), are known to aggressively charge the web or spiders directly to flush and chase the spider. However, these groups seldom prey on Theridiidae. On the other hand, *Chalybion* seems to have evolved aggressive mimicry against spiders of both Araneidae (Blackledge & Pickett 2000, Coville 1976) and Theridiidae (Uma & Weiss 2012). *Chalybion californicum* (Saussure, 1867) climbs the cobweb to gain access to the prey spider, *Parasteatoda tepidariorum* (see the web sketch in Fig. 4b), as well as following the dropping spider, or touches and vibrates gum-footed vertical line(s) from the bottom of the web to entice the spider (Uma & Weiss 2012), as with *Z. albicoxa* (Fig. 4b). These records suggest that efficiently utilising Theridiidae involved the evolution of specific modes of offensive behaviour to cope with the complex 3D web architecture, such as creeping-up and aggressive mimicry, as seen in both *Chalybion* and *Zatypota*. However, only *Z. maculata* has efficiently coped with the knockdown 3D web by means of a diving-style of aggressive imitation of prey.

## Ovicide by *Z. maculata*

Several polysphinctines are known to remove previous occupants on a spider host (infanticide) when encountering an already parasitised spider. In all cases, this was accomplished by means of rubbing movements of the ovipositor (Eberhard 2000, Gonzaga & Sobczak 2007, Takasuka & Matsumoto 2011a). Infanticide by removal would have evolved *de novo* among polysphinctines because the immediate outgroup of the group is comprised of parasitoids of egg-sacs or egg-nests of spiders (Matsumoto 2016), which seem unable to remove previous occupants enclosed by an egg-sac or egg-nest. In a quantitative study of polysphinctine infanticide by Takasuka and Matsumoto (2011a), ovipositing *Z. albicoxa* always removed any previous occupants regardless of the time costs or instar of the previous occupant because only one larva can complete its development even when superparasitism occurs (the opponent will be discarded immature with the spider carcass). This determination to commit infanticide results in superparasitism being very rare in this species; superparasitism was not observed among 201 immature *Z. albicoxa* individuals attached to spiders from egg to penultimate instar larva (Takasuka & Tanaka 2013).

Although it was only two trials, two *Z. maculata* also committed ovicide through the removal of an existing egg, as does *Z. albicoxa*, whereas both failed to detect the superparasitising larva. This indicates that once infant/ovicide is successfully accomplished, the behaviour is not repeated (although subsequent rubbing behaviour was observed), because in the field ovipositing females seldom encounter doubly parasitised spiders. However, this hypothesis needs to be quantitatively tested.

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## Legends of figures

**Figure 1.** Knockdown 3D web of *Nihonhimea japonica*. (a) The web constructed *in situ* (the campus of Kobe University) on *Osmanthus fortunei*. (b) The web reconstructed on a web substructure.

**Figure 2.** Sequence of offensive oviposition behaviour of *Zatypota maculata in situ*. (a) Clinging to one of the upper outermost supporting structures. (b) Creeping up on and facing the retreat in which the spider host hides. (c) Wasp and spider in a stalemate. (d) Grabbing the paralysed spider's abdomen (different individual).

**Figure 3.** *Zatypota maculata* lays an egg upon the abdomen of *Nihonhimea japonica* by means of the ventral-press *sensu* Takasuka *et al.* (2018).

**Figure 4.** Schemata of host theridiid webs utilised by three polysphinctines and ethogram of offensive oviposition behaviours of polysphinctines. (a) *Zatypota petronae* against *Theridion evexum* (Weng & Barrantes 2007). (b) *Zatypota albicoxa* against *Parasteatoda tepidariorum* (Takasuka et al. 2009, Takasuka & Matsumoto 2011b). (c) *Zatypota maculata* against *Nihonhimea japonica*.

**Supplementary video 1.** The typical creeping-style exhibited by wasp B. See the text for detailed explanation. The video was partly sped up by 30 times.

**Supplementary video 2.** The typical diving-style with ovicide upon a previous egg (1:07-1:30) exhibited by wasp B. See the text for detailed explanation.

**Supplementary video 3.** The diving-style (failure) and then the fall-back method of luring by aggressively climbing (success) with ovicide upon a previous egg (1:05-1:17) exhibited by wasp C. See the text for detailed explanation. The video was partly sped up by 30 times.

**Table 1** Known styles of offensive behaviour by polysphinctines and classification of their tactics

| species | spider host | web type | oviposition style | tactics classification | reference |
|---|---|---|---|---|---|
| *Zatypota maculata* | *Nihonhimea japonica* (Theridiidae) | knockdown 3D web | 1) creeping<br>2) diving into sheet | creeping-up<br>aggressive mimicry | this study |
| *Zatypota petronae* | *Theridion evexum* (Theridiidae) | 3D web with long viscid lines (Fig. 4a) | intruding into a retreat | abrupt plunge | Weng & Barrantes (2007) |
| *Zatypota albicoxa* | *Parasteatoda tepidariorum* (Theridiidae) | 3D web with gum-footed vertical lines (Fig. 4b) | 1) ambush (hanging from a vertical line)<br>2) alternative ambush (perching at a mid-height of the web)<br>3) climbing<br>4) reclining against a vertical line | aggressive mimicry<br>aggressive mimicry<br>creeping-up<br>aggressive mimicry | Takasuka *et al.* (2009), Takasuka & Matsumoto (2011b) |
| *Hymenoepimecis argyraphaga* | *Leucauge argyra* (Walckenaer, 1841) (Tetragnathidae) | horizontal orb web | plunging from above | abrupt plunge | Eberhard (2000) |
| *Hymenoepimecis veranii* | *Araneus omnicolor* (Keyserling, 1893) (Araneidae) | vertical orb web with a detached retreat and barrier web | plunging from barrier thread | abrupt plunge | Gonzaga & Sobczak (2007) |
| *Brachyzapus nikkoensis* | *Agelena silvatica* Oliger, 1983 (Agelenidae) | funnel sheet web | diving into sheet | aggressive mimicry | Iwata (1942), Matsumoto (2009) |
| *Polysphincta* sp. nr. *purcelli*<br>*Polysphincta janzeni* | *Cyclosa fililineata* Hingston, 1932 and *C. morretes* Levi, 1999 | vertical orb web | intentionally flushing a spider and then ambushing the returning spider | pure ambush | Kloss *et al.* (2016) |

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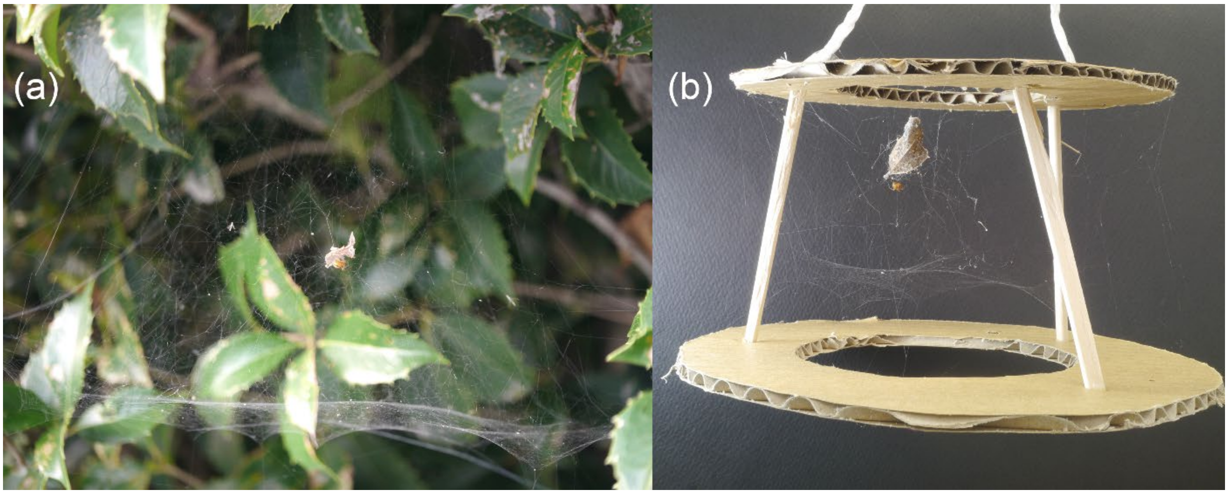
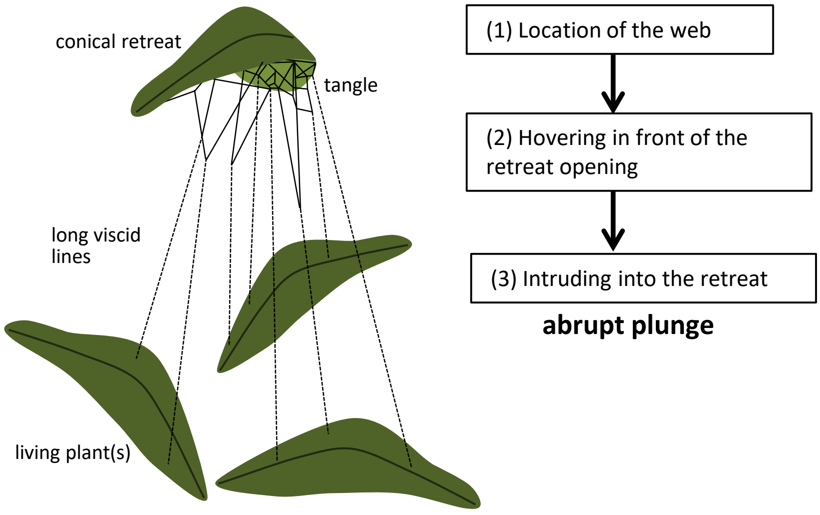


Figure 1 Takasuka *et al.*

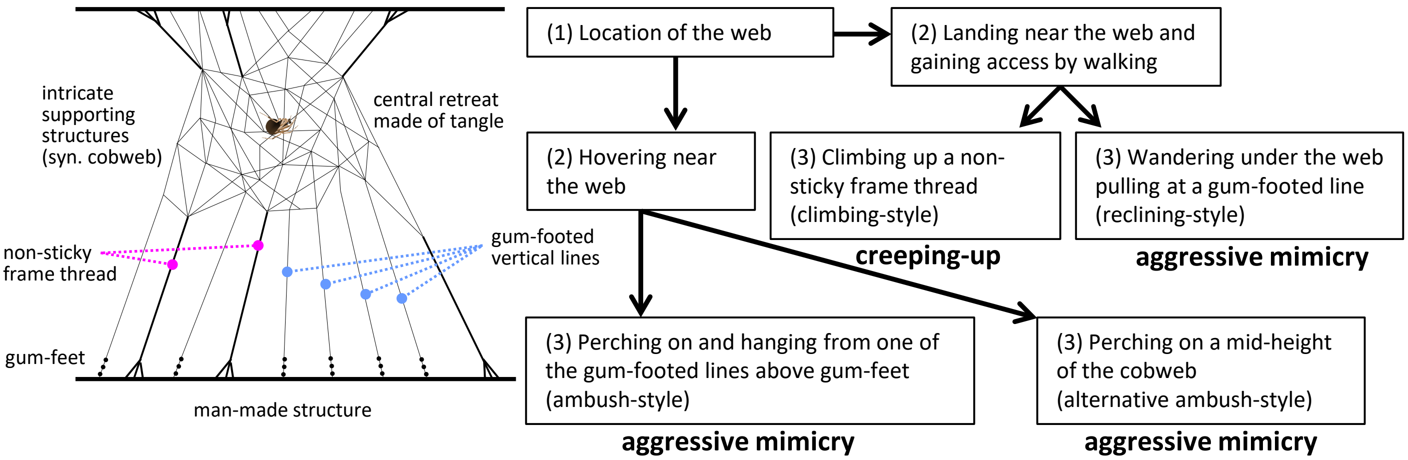
Figure 2 Takasuka *et al.*

Figure 3 Takasuka *et al.*

(a) *Zatypota petronae* against *Theridion evexum*
(Weng & Barrantes 2007)



(b) *Zatypota albicoxa* against *Parasteatoda tepidariorum*
(Takasuka *et al.* 2009, Takasuka & Matsumoto 2011b)



(c) *Zatypota maculata* against *Nihonhimea japonica*
(this study)

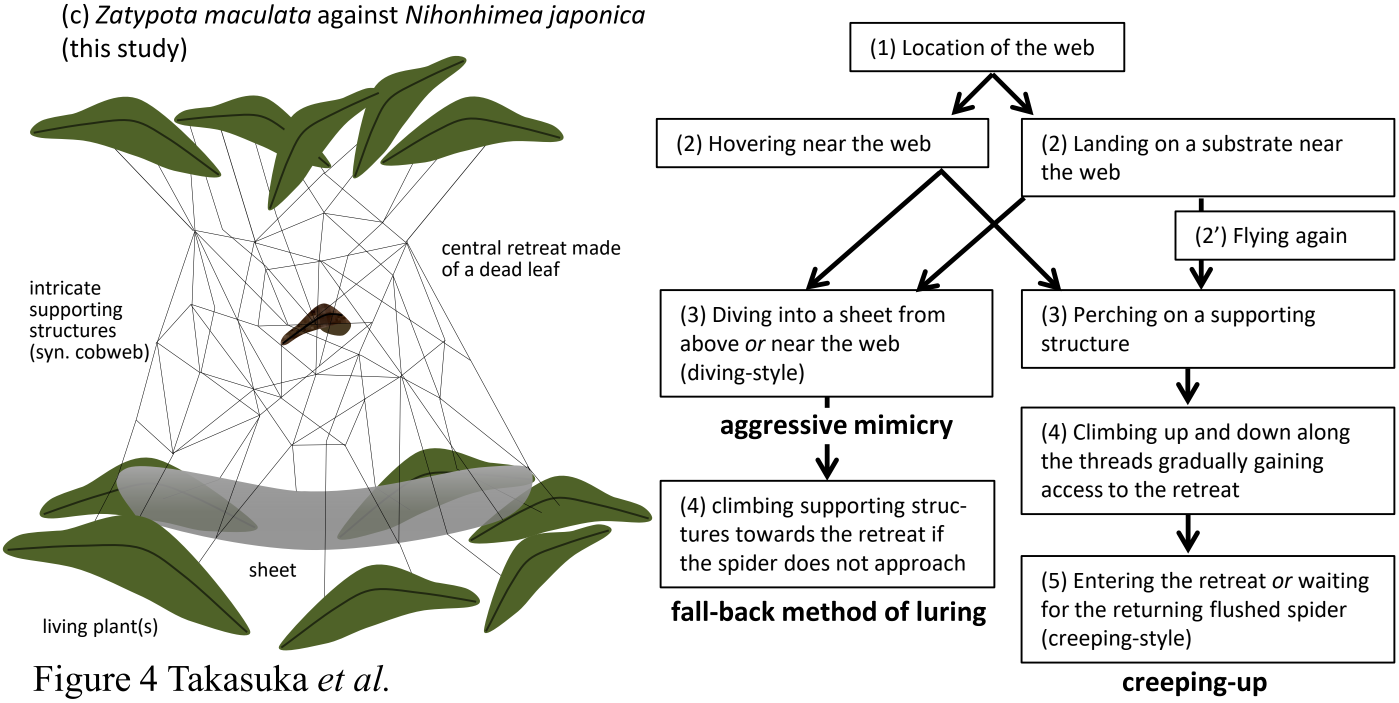


Figure 4 Takasuka *et al.*

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Video S1.** The typical creeping-style exhibited by wasp B. Seethe text for detailed explanation. The video was partly sped upby 30 times.
https://zslpublications.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fjzo.12668&file=jzo12668-sup-0001-VideoS1.mp4

**Video S2.** The typical diving-style with ovicide upon a previous egg (1:07–1:30) exhibited by wasp B. See the text for detailed explanation.
https://zslpublications.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fjzo.12668&file=jzo12668-sup-0002-VideoS2.mp4

**Video S3.** The diving-style (failure) and then the fall-back method of luring by aggressively climbing (success) with ovicide upon a previous egg (1:05–1:17) exhibited by wasp C. See the text for detailed explanation. The video was partly sped up by 30 times.
https://zslpublications.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fjzo.12668&file=jzo12668-sup-0003-VideoS3.mp4