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Parasitoid genus-specific manipulation of orb-web host spiders (Araneae, Araneidae)

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Short title

Parasitoid genus-specific manipulation of orb-web spiders

Abstract

1. Araneid spiders of genus *Araniella* are attacked by three polysphinctine parasitoid wasps *Polysphincta boops*, *P. tuberosa* and *Sinarachna pallipes*. Here we studied the trophic niche of sympatrically occurring parasitoids and the host manipulation they induced. We aimed to identify whether the variation in host response to manipulation is due to differences among parasitoids or among host species.

2. We found that final instar larva forced the spider host to build 3D ‘cocoon web’ to protect the parasitoid during pupation. The behaviour of parasitoid larva and the induced modification of the web architecture differed between wasps of genus *Polysphincta* and *Sinarachna* but not among three spider species. The larvae of genus *Polysphincta* forced the spider host to build the ‘cocoon web’ with high thread density within which the pupa was positioned horizontally. The larvae of *Sinarachna* forced the spider host to build web with sparse threads and the pupa was positioned vertically in the middle of the ‘cocoon web’.

3. There seems to be an investment trade-off in parasitoid wasps: some species manipulate host to build dense protective web, while pupating in a sparse cocoon, others make the spider to produce sparse web but build dense pupa wall.

Keywords

Polysphinctini; parasitoid-host interaction; behavioural manipulation; web architecture

Introduction

Parasitic organisms, parasites and parasitoids, have often developed the ability to manipulate the host phenotype including its morphology, physiology and behaviour for their own benefit (Moore, 2002). The function of behavioural manipulation has been described in many parasite-host systems (e.g. Moore, 2002) and their evolutionary significance and adaptation significance were discussed by Poulin (1995; 2010) ; Moore (2013) and Maure *et al.* (2013).

The response to manipulation varies (Thomas *et al.*, 2005; Poulin, 2010). It can result either from differences among hosts or among parasitoids or from the interaction between both. For example, variability of the host response to the same parasitoid has been found in amphipods hosts. The freshwater amphipod *Gammarus pulex* (Linnaeus) is regularly infected with acanthocephalan parasite *Pomphorynchus leavis* Müller that use either fish or birds as final hosts. *Pomphorynchus leavis* induced photophilic reaction in *G. pulex*, but not in *Gammarus roeseli* Gervais (Tain *et al.*, 2007). The inability to manipulate the behaviour of the invasive species *G. roeseli* might be explained by tight adaptation of the parasite to the local host species. The host response in this system varied even among populations as some parasitoid individuals induced a strong manipulation whereas others failed to do so (Franceschi *et al.*, 2010). Eberhard (2010) observed variability in the behavioural response of the spider host *Anelosimus octavius* Agnarsson after manipulation by polysphinctine wasp *Zatypota solanoi* Gauld. All parasitised spiders have been manipulated to build the safe retreat for larva pupation. But some manipulated spiders built a retreat for parasitoid larva using only silk threads, other incorporated leaves to improve the retreat. Eberhard (2010) suggested that this variability is at higher rather than lower levels in the hierarchy of behavioural manipulation mechanisms because the larva's effect is fine-tuned to details of the host's natural history. Korenko & Pekár (2011) described a host-specific response in the theridiid

spider *Neottiura bimaculata* (Linnaeus) and *Theridion varians* Hahn attacked by *Zatypota percontatoria* (Müller). The first host species responded by building a dense web, whereas the other one responded by building a specific spherical web.

A few studies have been devoted to behavioural manipulation of orb-web building spiders (Eberhard, 2000*a, b*, 2001; Gonzaga et al., 2010; Gonzaga & Sobczak, 2007, 2011; Sobczak *et al.*, 2009). Several polysphinctine wasps of the genus *Hymenoepimecis* associate with tetragnathid spiders and induce the production of a ‘cocoon web’ that has changed design in the number of radii and the spiral, but 2-dimensionality of the web is retained (Eberhard, 2000, 2001*a,b*). A completely different manipulation, when the spider has been forced to change 2D structure to 3D, has been found in araneid and nephilid spiders (Gonzaga *et al.*, 2010; Gonzaga & Sobczak, 2011).

Here we focus on Polysphinctine wasps (Polysphinctini *sensu* Townes, 1969) that are koinobiont ecto-parasitoids of spiders and they are narrowly associated with biology of their spider hosts (Fitton *et al.*, 1987). The larva, attached to the dorsal side of the abdomen, develops while the spider continues foraging. Shortly before pupation, the parasitoid final instar larva manipulates the web-building activity of the host in order to construct safe shelter against natural elements and predators during the parasitoid pupation. The architecture of the innate orb web is changed and becomes stronger providing more durable support for the wasp’s pupa.

We selected a host-parasitoid system in which few parasitoid species have overlapping trophic niches, i.e. attack hosts of the same or closely related species. The parasitoid wasps, *Polysphincta boops* Tschek, *Polysphincta tuberosa* (Gravenhorst) and *Sinarachna pallipes* (Holmgren), are exclusive parasitoids of orb-web spiders of the family Araneidae. *Polysphincta boops* was documented to be associated with *Araniella cucurbitina* (Clerck) and *A. opisthographa* (Kulczyński). *Polysphincta tuberosa* and *S. pallipes* were recorded from

several araneid species, namely *Araniella* sp., *Araneus diadematus* Clerck, *Araneus quadratus* Clerck, *Agelenatea redii* (Scopoli) and *Zigiella* sp. (Fitton *et al.*, 1988; Aubert, 1969). The spider genus *Araniella* includes several species with sympatric distribution in Palearctic. These are common orb-web weaving spiders in the canopies, inhabiting similar microhabitats (tree-crowns, shrubs), building planar orb-web of similar architecture, and having similar life histories (Levi, 1974; Roberts, 1985).

We studied the trophic niche and the niche overlap of the three parasitoid species, and compared several traits of the manipulation induced by larva. Then we investigated whether the variation in host response to manipulation is due to differences among parasitoids or among host species.

Methods

Frequency of parasitised spiders within communities of orb-web weaving spiders (Araneidae) were investigated in four sites: (1) in a commercial apple orchard in Starý Lískovec (district Brno, Czech Republic, 49°09'38"N, 16°33'38"E) in 2007 and 2008; (2) at a forest edge of Velké Lúky (Motešice, district Trenčín, Slovakia, 48°51'34"N, 18°13'45"E) during May and October 2012; (3) in an organic apple orchard in Bottonasco (Province of Cuneo, Italy, 44°24'47"N, 7° 24'43"E) in November 2009 – 2011; and (4) at a forest edge in Monterosso Grana (Province of Cuneo, Italy, 44°24'20"N, 7°19'17"E) in November 2011. At each site the spiders were collected by beating tree branches with a square shaped beating net (1 m² area) placed beneath the tree crown. At least 3 samples (each sample was made of beating 30 trees) were taken per site. Collected spiders were identified to generic level using Nentwig *et al.* (2013) and Roberts (1985) and matched to the species based on formerly occurring species in investigated localities (Isaia *et al.*, 2010; Korenko *et al.*, 2011, 2012; Korenko, unpub.) and on rearing collected specimens to adulthood. The host range of the parasitoids was estimated as

niche breadth using Smith's formula, FT , which unlike other commonly used measures is less sensitive to selectivity of rare resources and has good statistical properties (Smith, 1982).

Estimated host ranges were compared among parasitoids based on the z statistics.

Live parasitised and unparasitised spiders collected in the four sites above plus in two additional ones: (1) in forest in Aisone (Province of Cuneo, Italy, 44°17'29'N, 7°12'26'E) in November 2010; and (2) private garden in Hradečno (district Kladno, Czech Republic, 50°11'15'N, 13°59'16'E) in June 2012, were reared and observed in the laboratory. Web architecture of parasitised or non-parasitised spiders was studied by placing spiders individually to experimental arenas (100 x 100 mm base, 130 mm height) with an installed twig providing the space for spider web. Spiders were kept at a room temperature of $22 \pm 3^\circ\text{C}$, natural L:D regime and fed with a surplus of fruit flies (*Drosophila melanogaster* Meigen). The web building activity of parasitised spiders ($N = 29$) has been recorded until the larva killed and consumed the spider, and built the cocoon. In case of non-parasitised *Araniella* spiders ($N = 20$), these were observed for 20 days. The web-building activity has been recorded in 1-2 days period.

Hatched wasps were identified using Fitton *et al.* (1988) and Zwakhals (2006). The nomenclature of the polysphinctines follows Fitton *et al.* (1988) and Yu & Horstmann (1997). Voucher specimens are deposited in the collection of Kees Zwakhals (Netherlands), of Kamil Holý (Crop Research Institute, Prague, Czech Republic), and of the first author.

The web architecture of both parasitised and unparasitised spiders have been recorded: presence/absence of an orb web in the experimental arena had been recorded in 1-2 day interval. Spider spinning activity have been recorded using a digital camera Canon EOS 500 with an objective EFS 18-55 mm DC III and a macro objective EF 100 mm f/2,8L IS USM.

Body size of live spiders was measured using a scale paper placed under a transparent Petri dish with the precision of 0.5 mm. The angle of pupa orientation was measured by

transparent goniometer from photographs. The density of threads in the spider web of each parasitised individual and density of the threads in the wall of wasp cocoon was measured using SigmaScan Pro, Image Analysis V. 5.0.0 (SPSS Inc., Chicago, IL). The standardised photographs of the “cocoon web” and the wasp cocoons (4752 x 3168 pix) were transformed to 1 bit black and white mode (art line style) by Corel Photo Paint software v. 11 (Corel Corporation). Square-shaped sectors (200 x 200 mm) were cut out from the several sectors of the pictures. We estimated density of the threads from two different sectors of the web: 1) from the pupa surrounding and 2) from a distant part of web (sectors far away from pupa). A sector from the pupa surrounding was the place where one side of the square was touching the edge of pupa cocoon. A sector from a distant part of web was at least 200 mm away from pupa cocoon edge, but only in places with webbing. White area of silk threads in a picture was measured as a proportion (of the whole area). Density of silk threads of the web was compared between two sectors for each individual and among particular individuals. The cocoon was halved longitudinally using a blade. Images of the cocoon were taken, standardised and analysed similarly as mentioned above. The density of threads in the cocoon wall was compared among wasp species.

All analyses were performed within R environment (R Core Team, 2012). Linear Model (ANOVA) or Generalised Linear Model with binomial (GLM-b) or gamma (GLM-g) error structure was used to study the effect of the spider species and the wasp species on the selected traits of manipulation. ANOVA was used when the errors showed normal distribution and homoscedastic variance (spider body size). GLM-b was used when the response was binary and GLM-g was used when the response was continuous but showed heteroscedastic variance. Sum of squares of the type II were used to assess the effects of the two factors as the design was not orthogonal due to missing factor combinations (Pekár & Brabec, 2009). The density of cocoon wall was near to 1, therefore the data were angularly

transformed in order to homogenise variance and stay within 0 and 1 bounds. As the measurements performed on the cocoon were not statistically independent, there were repeated measurements per cocoon, the data were compared with Generalised Least Squares (GLS) from the nlme package (Pinheiro & Bates, 2000). GLS models population average (or marginal) relationship between study variables and takes into account correlation among measurements by correcting the variances. The correlation structure among measurements was specified as exchangeable due to only two repeated measurements on each cocoon

Results

Parasitism frequency and host specificity

In total 417 araneid orb-web spiders were collected in the four investigated sites (Table 1). Of these three genera, *Araniella*, *Mangora* followed by *Araneus*, were dominant. Only two genera *Araniella* and *Araneus* were parasitised. Juvenile spiders of the genus *Araniella* were parasitised with frequency between 4 – 14%, while juvenile spiders of the genus *Araneus* were parasitised only with frequency of 3%. Three species of the genus *Araniella* were parasitised with varying frequency: *A. cucurbitina* – 4 %, *A. displicata* – 14%, and *A. opisthographa* – 7.3% (pooled for two sites).

Three polysphinctine wasp species *Polysphincta boops* Tschek, *Polysphincta tuberosa* (Gravenhorst) and *Sinarachna pallipes* (Holmgren) were reared from *Araniella* and *Araneus* spider hosts (Table 2). The host range of the three species differed. *Polysphincta boops* parasitised only *A. opisthographa* (100%). *Polysphincta tuberosa* parasitised *A. cucurbitina* (13.3%), *A. opisthographa* (80%), and *Araneus diadematus* (6.7%). *Sinarachna pallipes* parasitised *A. cucurbitina* (17%), *A. displicata* (50%) and *A. opisthographa* (33%). Thus the host range was narrower in *P. boops* ($FT = 0.66$) and wider in *P. tuberosa* (0.87) and *S. pallipes* (0.90), but not significantly different among species ($z < 0.9$, $P > 0.39$, Fig. 4B).

The body size of parasitised spider hosts were as follows: *A. diadematus* (mean = 3 mm), *A. cucurbitina* (3.3), *A. displicata* (3.4) and *A. opisthographa* (3.4). The difference in body size between spider hosts was not significant (ANOVA, $F_{3,18} = 0.18$, $P = 0.91$): it was on average 3.3 (SE = 0.11).

Web architecture manipulation

Unparasitised spiders of *Araniella opisthographa* (N = 8), *A. displicata* (N = 12) and *A. cucurbitina* (N = 5) constructed only 2-dimensional webs during 20 days of observation. The web architecture was similar among species (Fig. 1). *Araniella* spiders were observed in the field to build 3-dimensional web structure only when a female guarded its cocoon (N = 4).

Final instar larva of all three wasp species induced production of a unique 3-dimensional ‘cocoon web’ in its spider host, but considerable differences were observed in the architecture (Fig. 2). The difference between web density surrounding pupa and outer part of web varied significantly among wasp species (GLS, $F_{2,16} = 7.0$, $P = 0.007$, Fig. 3A), but not among spider species (GLS, $F_{2,16} = 1.7$, $P = 0.22$). The density of threads in a web induced by *S. pallipes* did not differ between the pupa surrounding and the outer parts of web (contrasts, $P > 0.38$). But pupas of *P. boops* and *P. tuberosa* were surrounded by significantly more dense silk around the pupa than at outer parts of web (contrasts, $P < 0.01$). The differences between *A. opisthographa* and *A. displicata* and between the two *Polysphincta* species were not significant (contrasts, $P > 0.1$).

The density of threads surrounding the pupa differed significantly among wasp species (GLM-g, $X^2_2 = 16.5$, $P = 0.0003$, Fig. 3B), but not among spider species (GLM-g, $X^2_2 = 1.1$, $P = 0.57$). The density of threads around *Sinarachna* pupa was on average 17.6% (SD = 4.6), while that of *Polysphincta* was on average 39.4% (SD = 18.0). There was no significant difference between *P. boops* and *P. tuberosa* (contrast, $P = 0.43$).

Pupa

Following manipulation the final instar larva pupated at the same place where she killed the spider. The angle at which pupa was oriented differed significantly among wasp species (ANOVA, $F_{2,16} = 137.9$, $P < 0.0001$, Fig. 4A), but not among spider species (ANOVA, $F_{2,16} = 0.5$, $P = 0.65$): pupa of *S. pallipes* was vertically oriented at average angle of 81° (SD = 10.5), whereas pupa of *P. boops* and *P. tuberosa* was horizontally oriented at average angle of 172.5° (SD = 11.5). There was marginally significant difference between *P. boops* and *P. tuberosa* (contrast, $P = 0.047$). The place where pupa was produced differed significantly among wasp species (GLM-b, $X^2_2 = 11.3$, $P = 0.003$), but not among spider species (GLM-b, $X^2_2 = 4.5$, $P = 0.1$). The cocoon of *Polysphincta* pupas were built inside of the 3D web surrounded by dense tangle (55%, $N = 11$) or outside the main web. These pupae were built at the base of tree twigs (27%) or in a corner of the experimental arena (18%). The pupa of *Sinarachna* always took position at the middle of web (100%, $N = 11$). There was no significant difference between *P. boops* and *P. tuberosa* (contrast, $P = 1$).

The cocoons enclosing pupa were fusiform, diaphanous white or yellowish, with open and sparse construction in *Polysphincta* (Fig. 5A, B) and narrowly fusiform, light brownish – yellowish, and densely woven with a tight outer cover of coarse fibres in *Sinarachna* (Fig. 5C). There was a significant difference among the thread density of cocoon wall of three wasp species (GLS, $F_{2,13} = 173$, $P < 0.0001$): the cocoon wall of *S. pallipes* was significantly more dense than those of both *Polysphincta* species, and the cocoon wall of *P. tuberosa* was significantly more dense than that of *P. boops* (contrast, $P < 0.0001$, Fig. 4C).

Discussion

We found that investigated *Polysphincta* and *Sinarachna* wasps are associated with spiders of the genus *Araniella*, in particular. The wasps have been reported to associate with other areneid spider hosts (e.g. Gauld & Dubois, 2006; Fitton *et al.*, 1988, 1987; Aubert, 1969), but records are sporadic and some of them should be revised. *Araniella* spiders were most frequently attacked by parasitoids likely because they were the most dominant species of orb-web weaving spiders in the study spider communities. In all cases, the most abundant species from the spectrum of available hosts was the most parasitised one. This agrees with a former study (Korenko *et al.*, 2011), where *Zatypota percontatoria* (Müller) wasp preferred the most abundant host species from a spectrum of several suitable hosts, and was able to shift the host based on its quality and abundance. Although there are old notes on *P. boops* and *S. pallipes* to attack spiders of the family Theridiidae (3D web weavers) (Brischke, 1877; Nielsen, 1928), it is likely misidentification due to pronounced difference in life history and web architecture (2D versus 3D web). This is supported by our field observations (Korenko, unpub.) as no case out of 108 theridiid spiders found in Bottonasco was parasitised by the genus *Polysphincta* and *Sinarachna*. Theridiid spiders are parasitised by wasps of genus *Zatypota*, instead (Korenko & Pekár, 2011; Korenko, unpub.). The polysphinctines must have evolved narrow association fine-tuned to host biology (foraging, web building, antipredatory behaviour) to effectively use specific host biological traits (e.g. behavioural and physiological) for their benefit. They evolved adaptations to attack the 2D web. Their attack strategy is so specific (Takasuka & Matsumoto, 2011) that a parallel association with 3D web weavers (Theridiidae) is unlikely.

The study of the web-building activity of three *Araniella* species (*A. displicata*, *A. cucurbitina* and *A. ophistographa*) revealed that non-parasitised spiders build 3D web only as adult females to guard its cocoon. Parasitoids thus might be evoking an innate behaviour in

the manipulated host. The behaviour might be naturally used only by adult females, not by juveniles which are parasitised and killed before reaching adulthood.

We studied several traits of web-manipulation. A few architectural characteristics of the induced ‘cocoon web’ were similar between manipulated spider hosts: 2D web was changed to 3D structure, and absence of the sticky threads. In other traits, such as thread density, or the pupa orientation, the hosts differed. The variability was explained by the wasp species, not by the spider species. More specifically, wasps of the genus *Polysphincta* manipulated spider host in a different way than *S. pallipes* wasps. For example, all manipulated spiders have been forced to find some “safe” place, a place protected from all sides by spider silk or a habitat structure, such as tree twig, in the provided space, but position of this safe place differed. Spiders manipulated by *Sinarachna* chose the safest place in the centre of web, whereas spiders manipulated by *Polysphincta* chose place beneath base of tree twigs or in the corner of experimental arena. How could such difference be explained? We presume that the two parasitoid genera use different chemicals (e.g., neuromodulators), which evoke specific changes in *Araniella* host behaviour. The lack of difference between two *Polysphincta* species is likely due to their phylogenetic relatedness as compared with *Sinarachna*. According to phylogenetic analysis (Gauld & Dubois, 2006), wasps of genus *Polysphincta* belong to the taxonomical clade ‘F’, which also includes species of the genera *Acrotaphus* and *Hymenoepimecis*. The manipulation behaviour observed in these wasps has similar characteristics to that by *Polysphincta* (Gonzaga *et al.*, 2010; Gonzaga & Sobczak, 2011; this study), but differs considerably from manipulations induced by wasps of other taxonomic clades (Korenko, unpub.).

Overall the three wasp species use a different strategy for protection of themselves during pupation. The pupa of *Polysphincta* is protected by dense tangled silk threads produced by the manipulated spider. The wall of pupa cocoon is thinner and sparser. On the

contrary, the pupa of *S. pallipes* is surrounded by fewer threads produced by the spider, but is enclosed in a cocoon with a strong wall. So there seems to be a negative correlation between the web and cocoon density. Such correlation is achieved via trade-off in investment to host manipulation *versus* the cocoon production. It seems that both strategies are similarly effective in protection. A comparative study is needed to explain whether the lack of host manipulation as shown by *S. pallipes* led to evolution of stronger protection by the wasp larva, or advanced host manipulation as shown by *Polysphincta* has led to evolution of weaker pupa protection.

Poulin (1995) set up four criteria for consideration of behaviour manipulation to be adaptive in parasites: (1) they must be complex, (2) they must show signs of a purposive designs, (3) they arose independently in several lineages of hosts or parasites and (4) they must shown to increase the fitness of the parasite. All these criteria seem to be satisfied for the web architecture manipulation induces by polysphinctine parasitoids, though it has not been demonstrated yet trait this is adaptive. Evidence for increasing survival of parasitoid offspring induced by host's behaviour was reported by Matsumoto (2009). He observed that the induced webbing had been protecting parasitoid larva against ant predators. Similar induction of 3D web in the pupa surrounding, as reported here, was also reported for acrocerid parasitoids (Kehlmaier et al., 2012). So far it is not known whether modification of spider web architecture is effective against enemies of polysphinctines, including hyperparasitoids. We plan to address this topic in future. The *Sinarachna* genus belongs to clade 'D' which includes wasps of the *Sinarachna/Reclinervellus* genus complex (Gauld & Dubois, 2006). *Sinarachna* induced considerably different manipulation of spider architecture when compared to those of the genus *Reclinervellus*. *Sinarachna* induced change of 2D web to 3D symmetric structure where the pupa hangs in its centre; *Reclinervellus* only rebuilds and reinforces the innate 2D web and pupa is nested in this 2D structure (e.g. Matsumoto & Konishi, 2007). This marked

difference in the web architecture change induced by parasitoid larva inside one clade might be explained as follows: *Sinarachna* is associated to hosts with different life history (including web architecture and antipredatory behaviour) than *Reclinervellus*. *Sinarachna* is attacking Palearctic 2D web weavers which do not use stabilimentum (a conspicuous [silk](#) structure in the spider web having a variety of shapes (Foelix, 1996). On the contrary, the *Reclinervellus* is mostly tropical genus associated with 2D web weavers of genera *Cyclosa* and *Allocyclosa*, which use the stabilimentum as a camouflage protecting spiders against potential predators (Neet, 1990). Incorporation of the parasitoid pupa onto the web with stabilimentum provided protection for wasp pupa as well (Matsumoto & Konishi, 2007). The trophic niche of the three investigated wasp species overlapped both in terms of taxonomy and body size of hosts. The overlap in size is given by similar body size of the parasitoids (Fitton *et al.*, 1988). The host spectrum is also restricted taxonomically in other related wasp species. The small genus *Acrotaphus* is associated with *Argiope* (Araneidae) (Gonzaga & Sobczak, 2011; Eberhard, unpub.). The species rich genus *Hymenoepimecis* is associated with wider spectrum of hosts from families Nephilidae, Tetragnathidae and Araneidae, but their association is strictly species specific. *Nephila clavipes* Linnaeus is attacked by *H. robertsae* Gauld and *H. bicolour* (Brulle) (Gonzaga *et al.*, 2010). Tetragnathid genus *Leucauge* is attacked by *H. japi* Sobczak *et al.* (*L. roseosignata* (Mello-Leitão)), *H. argyraphaga* Gauld (*L. argyra* (Walckenaer)) and by *H. tedfordi* Gauld (*L. mariana* (Taczanowski)) (Sobczak *et al.*, 2009; Eberhard 2000a,b, 2001; personal com.) and *Araneus omnicolor* (Keyserling) is attacked by *H. veranii* Loffredo & Penteado-Dias (Gonzaga & Sobczak, 2007). However, spiders of all three families are orb-web weavers and the general architecture of their innate web is similar, the changes in web architecture induces by particular *Hymenoepimecis* differ considerable. Nephilids are forced to build 3D tangle web, tetragnathids are forced to build cocoon web in 2D and araneids are forced to add lines to the innate 2D web and transform it

to 3D tangled structure (Eberhard and Gonzaga, personal com.). Manipulation of web architecture induced by investigated wasps seems to be the most similar to manipulation induced by nephilids. We hypothesize that this discrepancy between taxonomy and behaviour could be a consequence of higher importance of the host's natural history than taxonomical relatedness.

The manipulations induced in web-building spiders reported so far appear to have common the adaptive value – silk threads in different shapes are providing shelter for parasitoid pupa against environment and enemies. We need more examples on behavioural manipulation in spiders by polysphinctine species in order to understand the evolution of host-parasitoid system in polysphinctines.

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Table 1. List of spider host species on four sites with their relative frequency of abundance.

Number of parasitised hosts is given following a slash.

Species	Site			
	Bottonasco (I)	Monterosso Grana (I)	Starý Lískovec (CZ)	Veľké Lúky (SK)
<i>Aculepeira ceropegia</i> (Walckenaer)	0	0	0.01	0
<i>Agalenate redii</i> (Scopoli)	0.01	0	0.02	0
<i>Araneus alsine</i> (Walckenaer)	0	0	0	0.03
<i>A. diadematus</i> Clerck	0.02	0.02	0	0.2
<i>Araneus</i> sp.	0.03	0.04 / 1	0.11	0
<i>Araniella cucurbitina</i> (Clerck)	0	0	0.24 / 2	0
<i>A. displicata</i> (Hentz)	0	0.86 / 6	0	0
<i>A. opisthographa</i> (Kulczyński)	0.67 / 5	0	0	0.74 / 2
<i>Cyclosa conica</i> (Pallas)	0.15	0.02	0.01	0
<i>Gibbaranea bituberculata</i> (Walckenaer)	0.01	0.02	0.01	0.03
<i>Mangora acalypha</i> (Walckenaer)	0.1	0.02	0.58	0
<i>Singa hamata</i> (Clerck)	0	0	0.02	0

<i>Zilla diodia</i> (Walckenaer)	0.01	0.02	0	0
Total individuals	110	50	227	30

Table 2. Number of cases of parasitised araneid spider species by three wasp species and the sites, where these were collected. The wasp individuals were reared to adulthood in order to record details of manipulation and to identify them to species.

Spider host	Parasitoid		
	<i>P. boops</i>	<i>P. tuberosa</i>	<i>S. pallipes</i>
<i>Araneus diadematus</i>	–	Hradečno (N = 1)	–
<i>Araniella cucurbitina</i>	–	Starý Lískovec (N = 2)	Aisone (N = 2)
<i>A. displicata</i>	–	–	Monterosso (N = 6)
<i>A. ophistographa</i>	Bottonasco (N = 2)	Veľké Lúky (N = 12)	Bottonasco (N = 4)

Figures

Fig. 1. Innate 2-dimensional web of *Araniella displicata* spiders built in the laboratory. Scale = 1 cm.

Fig. 2. A. Cocoon web of *Araniella ophistographa* induced by *Polysphincta tuberosa*. Insert: *P. boops*. B. Cocoon web built by spider host *Araniella displicata* under manipulation by *S. pallipes*. Scale = 1 cm.

Fig. 3. Comparison of the cocoon web architecture. A. Difference between thread density surrounding pupa and the web margin. B. Thread density surrounding pupa. Points are means, whiskers are 95% confidence intervals.

Fig. 4. Comparison of the pupa traits. A. Angle of orientation. B. Relative host frequency of the three wasp species. C. Silk density of cocoon wall. Points (lines or bars) are means, whiskers are 95% confidence intervals.

Fig. 5. Cocoon wall of *P. boops* (A), *P. tuberosa* (B), and *S. pallipes* (C). Scale = 0.1 mm.