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Predatory wasps learn to overcome the shelter defences of their larval prey

MARTHA R. WEISS*, ERIN E. WILSON* & IGNACIO CASTELLANOS†
*Biology Department, Georgetown University
†Department of Entomology, University of Maryland, College Park

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Larvae of *Epargyreus clarus* (Hesperiidae), the silver-spotted skipper, inhabit leaf-and-silk shelters that they construct on their leguminous host plants. In the field, *Polistes* spp. (Vespidae) wasps land on the shelters, quickly extracting and killing the larvae within. In marked contrast, wasps that emerge from field-collected colonies maintained in the laboratory visit and examine leaflets bearing sheltered caterpillars, but only rarely do they extract and kill the sheltered larvae. To examine whether learning is involved in the development of the ability of *Polistes* wasps to forage successfully on sheltered *E. clarus* larvae, we tested the responses of *P. fuscatus* and *P. dominulus* wasps to sheltered *E. clarus* larvae before and after their exposure to unsheltered larvae that were visible either on an opened host-leaf shelter (*P. fuscatus* and *P. dominulus*) or on a nonhost leaf in the absence of a shelter (*P. fuscatus*). After killing and processing an unsheltered larva that was visible on an opened leaf shelter, a majority of foragers subsequently extracted and killed larvae from closed shelters. Wasps that killed and processed an unsheltered larva on a nonhost leaf, on the other hand, generally did not later open shelters. Thus, it seems that experience with an exposed larva in the context of its shelter is necessary for a wasp to be able to prey on sheltered larvae. We conclude that the wasps must learn to associate the taste of the larva with shelter-related cues, such as presence of leaf damage and silk. In nature, this initial exposure may occur when the larva is visible in or near its shelter, perhaps when feeding or constructing a new shelter. Learning opportunities will thus depend on larval density. Our results show that invertebrate predators can learn to overcome their prey's defences, and are therefore able to make use of previously inaccessible prey.

Learning, broadly defined as modification of behaviour as a result of experience (Papaj & Prokopy 1989; Cunningham et al. 1998; Morse 2000), can contribute to a predator's ability to respond to the defences of its potential prey. Learning can result in an increase in foraging success over a predator's lifetime via a decrease in prey location and handling time, or an increase in capture success (Takagi et al. 1980; Cunningham & Hughes 1984; Daniel & Bayer 1987; Rovero et al. 1999; Hughes & O'Brien 2001). Predators can also learn to recognize acceptable novel prey, thereby increasing diet breadth (Hughes & Dunkin 1984; Pasteels & Gregoire 1984; Blois & Cloarec 1985; Persons & Rypstra 2000). In addition, predators can learn to identify and avoid noxious or toxic prey items, sometimes after only a single encounter (Berenbaum & Miliczky 1984; Dejean 1988). Here we report on an invertebrate predator that learns to overcome the defences of a shelter-building caterpillar, a prey item that is initially inaccessible.

Caterpillars in at least 18 families construct and inhabit leaf shelters that can afford them protection from both vertebrate and invertebrate predators (Damman 1987; Loeffler 1996; Eubanks et al. 1997; Jones et al. 2002). We have observed predatory *Polistes* spp. wasps in the field land on leaf shelters made by larvae of *Epargyreus clarus*, the silver-spotted skipper, and adeptly kill and process the larvae hidden within. We found, however, that naïve foragers did not attack or kill the sheltered caterpillars, even after many hours of exposure (Jones et al. 2002). As predatory wasps are known to be capable learners (O'Donnell & Jeanne 1992; Collett 1995; McPheron 1996; Shafir 1996; Raveret Richter 2000), we hypothesized that learning was involved in the development of the wasps' ability to forage successfully on sheltered *E. clarus* larvae. Wasps have been observed to target and open leaf shelters and galls made by lepidopteran larvae (Steiner 1984;

Correspondence and present address: M. R. Weiss, Biology Department, Georgetown University, 406 Reiss Building, Washington, D.C. 20057-1229, U.S.A. (email: weissm@georgetown.edu). I. Castellanos is now at the Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Hidalgo, Apartado Postal 69-1, Pachuca, Hidalgo 42001, México.

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Raveret Richter 1988), and although it has been suggested that this behaviour may be based on learning, the idea had not been examined experimentally.

To assess the importance of learning in the development of an ability to extract *E. clarus* larvae from their shelters, we followed a cohort of predatory *Polistes* wasps through a three-part protocol (Fig. 1). In the first phase ('pre-experience'), we assessed the response of naïve wasps to sheltered larvae. In the second phase ('experience'), we allowed the same individuals to kill and begin to process an unsheltered larva, either in the context of an opened shelter, or away from the shelter. In the third phase ('postexperience'), we again exposed the wasps, now experienced, to sheltered caterpillars to determine whether their behaviour had changed in comparison to their behaviour when naïve. For wasps that were able to remove caterpillars from shelters, we determined whether the time required to extract the caterpillar decreased with increased experience, and assessed the consistency of extraction methods over time.

METHODS

Study Taxa

Polistes *spp.*

The paper wasp genus *Polistes* (Vespidae) is comprised of about 200 species, with worldwide distribution (Akre 1982). *Polistes fuscatus* Fabricius is native throughout North America (Krombein et al. 1979), while *Polistes dominulus* Christ, an Old World paper wasp, was accidentally introduced into the U.S. in the late 1970s (Gamboa et al. 2002) and is now common in the Washington, D.C. area. Both species have an annual colony cycle, with nests founded by one or more overwintered mated females in early spring (Turillazzi & West-Eberhard 1996). The adults feed on floral nectars and other sweet exudates, but catch and process caterpillars and other invertebrate prey to feed to the larvae back at the nest (Michener & Michener 1951; Raveret Richter 2000). Adults also consume some of the liquid from the killed prey (Michener & Michener 1951).

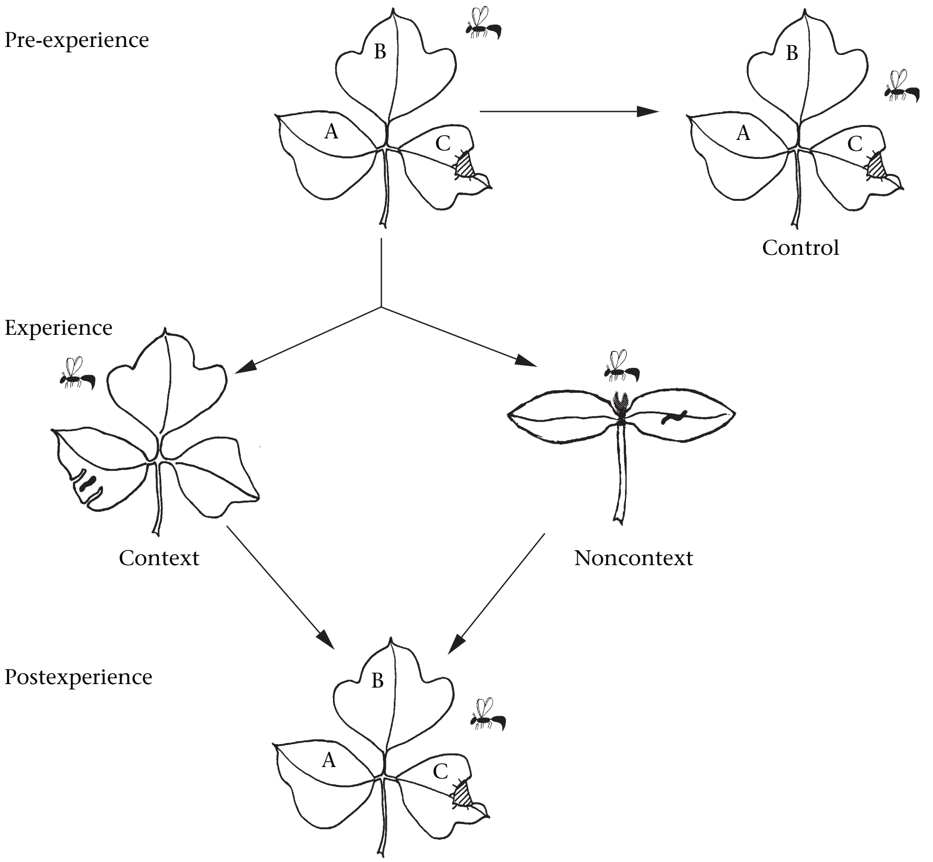

Figure 1. Experimental design for *P. fuscatus* trials. In the 'pre-experience' phase, we exposed naïve wasps to a host leaf on which an *E. clarus* larva had built a shelter, and assessed the wasps' behaviours with respect to the sheltered larva (on leaflet C) and the other two leaflets (A, B). Following at least 3 h of 'pre-experience', all wasps except those that had spontaneously extracted and killed a caterpillar during this phase went on to the next stage of the experiment. Two groups of wasps received 'experience' with *E. clarus*: 'context' foragers were allowed to kill and process a larva visible on its opened shelter ($N=16$ wasps), and 'noncontext' foragers were allowed to kill and process a larva visible on a nonhost leaf ($N=9$ wasps). A third group of foragers was given additional exposure to sheltered *E. clarus* larvae and served as a control ($N=15$ wasps). In the 'postexperience' phase, wasps that had received either context or noncontext experience were again exposed to sheltered larvae, and the responses of the wasps assessed.

We collected *P. fuscatus* nests from the field in Patuxent, Maryland, U.S.A., in early June 2001 and late May 2002, and collected *P. dominulus* from the campus of the University of Maryland, College Park, Maryland, in early June 2001. We collected small nests, generally containing a single foundress and numerous pupae, larvae and eggs, and attached each one with glue to its own T-shaped wooden support. *Polistes fuscatus* nests were kept at Georgetown University, either indoors (23 °C) in Plexiglas or mesh cages ($0.6 \times 0.6 \times 0.9$ m), or outdoors in 2-m^3 mesh field cages, with one nest to a cage. *Polistes dominulus* nests were maintained in a greenhouse at the University of Maryland at 26–30 °C inside a 2-m^3 Plexiglas cage, with two nests in the cage. All wasps were individually marked with coloured nontoxic ink as they emerged.

We provided each colony daily with live lymantriid and/or geometrid larvae on box elder (*Acer negundo*) leaves throughout the course of the experiment. When those larvae were scarce, we supplemented the wasps' diet with live wax moth larvae, *Galleria mellonella*, or freshly killed mealworms (*Tenebrio* sp.), which we also placed on the *Acer negundo* leaves. We generally followed the feeding equation estimated from Geitzenauer (1993) and Karsai & Hunt (2002), providing each nest with a standardized 0.25 prey item per wasp larva per day, so that nests were given more prey items as the colony grew. These larvae were placed in the cage in the afternoon, after the end of the day's testing. The cages contained fresh water and honey, as well as cardboard for nest construction.

Epargyreus clarus

The silver-spotted skipper, *Epargyreus clarus* Cramer (Lepidoptera: Hesperiidae), ranges throughout North America from Saskatchewan in the north to Baja California, Texas and Florida in the south (Scott 1986). In the Washington, D.C. area these large skippers fly throughout the summer and early autumn, and commonly use black locust trees, *Robinia pseudoacacia*, kudzu vine, *Pueraria lobata*, or other legumes as hosts (Clark & Clark 1951; Allen 1997). All *E. clarus* larvae construct and inhabit leaf-and-silk shelters (Fig. 2); they spend about 95% of the time resting on the ceiling inside their shelters, leaving only briefly to feed (about 4 min/h), or to construct a new shelter (Lind et al. 2001). Sheltered larvae are not generally visible from outside the shelter. *Epargyreus clarus* larvae reared on either kudzu or black locust do not seem to be chemically defended, as they are highly palatable to a range of invertebrate predators, including vespid wasps, ants, anthocorid bugs, lacewing larvae and salticid spiders, all of which are found on or around their host plants (unpublished data). To obtain larvae, we collected *E. clarus* butterflies on the campus of Georgetown University and placed them in an outdoor field cage (2-m^3) that contained freshly cut kudzu leaves for oviposition and abundant flowers for nectar foraging. Leaves bearing newly deposited eggs were collected daily, and emerged larvae were reared on kudzu leaves in the laboratory. Experiments were carried out daily between mid-June and mid-August, 2001 and 2002.

Figure 2. (a) Third-instar *E. clarus* shelter on kudzu, showing silken guy-wires that secure the shelter to the leaflet. A larva is resting on the 'ceiling' of the shelter, and is not visible from the outside. (b) An experienced *P. fuscatus* forager processing a caterpillar extracted from the shelter on the right.

Pre-experience

All naïve wasps were given 'pre-experience' exposure to quantify their response to sheltered *E. clarus* larvae: we placed kudzu leaves bearing individual sheltered caterpillars inside cages containing active *P. fuscatus* or *P. dominulus* colonies, and monitored the behaviours of the foragers for a minimum of three periods of 0.75–1.5 h (hereafter, a trial). The evening before a trial, we allowed a single second- or third-instar *E. clarus* larva to build a shelter on any one of the three leaflets of a fresh kudzu leaf, thereafter designated as the 'C' leaflet; the other two undamaged leaflets were arbitrarily designated 'A' and 'B'. At the start of a trial, we placed the test leaf with the larva upright in a small glass jar on the floor of the wasp cage, and arranged the leaf so that all leaflets were equally in the sun or the shade.

When several wasps were actively foraging, we removed as many as three foragers so that we could focus on a specific individual's behaviour. Wasps seemed unaffected by the removal, and behaved normally when returned to the cage. Using a stopwatch and either a scribe or a voice-activated tape recorder, we recorded the identity of foragers and the number and duration of any visits to a leaflet or shelter. We also noted whether the wasps successfully removed the caterpillars from their shelters. Those that did so during this 'pre-experience' period were termed 'spontaneous learners', and were not subsequently given any formal experience. Brief alights of less than 1 s were rounded to 1 s, and time spent grooming or basking on the leaf was not counted. Each wasp was presented with a sheltered caterpillar for at least 3 h, accrued over the course of several trials. Time elapsed between subsequent trials ranged from several hours on a single day to 3 days, depending on the individual wasp's activity. For each forager in a given trial, we calculated the time spent on each leaflet (A, B and C) as a percentage of total time spent on all leaflets. Time spent on the shelter itself was included in the total time spent on leaflet C. We then calculated the mean percentage of time spent on each leaflet for all trials by a given wasp, so that each wasp was represented once in the grand mean determined for all wasps. Trials in which a wasp spent less than 10 s on the leaflets were excluded from the analyses. We used repeated measures analysis of variance (ANOVA), in which 'leaflet' was the fixed factor, and individual wasp was the random factor, to compare the percentage of time spent by the wasps on shelter-bearing (C) leaflets against the percentage of time spent on each of the other leaflets (A and B). (We used a repeated measures ANOVA because each wasp contributed three scores to the ANOVA). Following a significant overall ANOVA, we used two-tailed paired *t* tests to compare the percentage of time spent on pairs of leaflets; a Bonferroni adjustment was used to control for possible type I error. For *P. fuscatus*, we similarly compared the percentage of total visits made on leaflets A, B and C.

Experience

Following at least 3 h of 'pre-experience', all wasps except those that had spontaneously extracted and killed a caterpillar in the first three pre-experience trials went on to the next phase of the experiment. One subset of foragers was given a 'context' experience with an *E. clarus* larva visible on its opened shelter, another subset was given a 'noncontext' experience with an *E. clarus* larva visible on a nonhost leaf or petri dish, and a third group of foragers was given additional exposure to sheltered larvae and served as a control. For 'context' exposures, we gently opened the shelter of a second- or third-instar larva on a kudzu leaf just prior to the start of the trial, so that the larva was fully visible on the exposed inner surface of the shelter. For 'noncontext' exposures, we placed a second- or third-instar larva either in the centre of a plastic petri dish (8.5-cm diameter) with a circle of green paper affixed below, or on the upper surface of a *Viburnum* sp. leaf. (We switched to the *Viburnum* leaf protocol because wasps rarely visited the petri dish.) The larva and its substrate (kudzu leaf, petri dish, or *Viburnum* leaf) were placed in or on a glass jar on the floor of the cage, as the sheltered larvae in the pre-experience trials had been. We removed other active foragers so that a focal wasp that had met the criterion of 3 h of exposure to sheltered caterpillars could encounter and kill a larva undisturbed. We then captured the wasp in a small plastic cup and removed her from the cage before she returned to the nest. We did this to prevent her from sharing the processed caterpillar with larvae and other foragers at the nest, in case such feeding experience influenced later foraging behaviours of the larvae or adult foragers (Rayor & Munson 2002). The forager was later released back into her cage without the processed larva. A wasp was considered 'experienced' after having killed and processed an *E. clarus* caterpillar. *Polistes fuscatus* wasps were given context experience in 2001, and both context and noncontext experience in 2002; *P. dominulus* wasps were given context experience in 2001; they did not receive noncontext experience.

Postexperience

To assess whether wasps, once experienced, responded differently to sheltered larvae than they did when naïve, we again exposed both context and noncontext wasps to a sheltered second- or third-instar *E. clarus* larva. Re-exposure usually took place the day after a wasp became experienced. We recorded the wasp's behaviour, including the number and duration of visits to leaflets A, B and C and the shelter, and noted the wasp's success or failure at extracting and killing the caterpillar. If a wasp killed the larva, we recorded how long she took to open the shelter and/or extract the caterpillar, and the method she used to do so. Extraction time was measured as the cumulative amount of time a forager was actively engaged in trying to extract the caterpillar, ending when she successfully killed it. Here again, we removed the wasp upon successful capture of the larva so she would not take the processed prey back to the nest. If an experienced wasp did not extract a sheltered larva after at least two postexperience trials, we allowed her to have another experience with an unsheltered caterpillar. The number of wasps that opened shelters in each treatment (context, noncontext and control) was compared using a two-by-three chi-square test of independence followed by two-way chi-square tests of independence where appropriate. A Bonferroni adjustment was used to control possible type I error.

Controls

Foragers of both *P. fuscatus* and *P. dominulus* that had been exposed to sheltered *E. clarus* larvae for three 'pre-experience' trials were exposed to sheltered larvae for several additional trials to control for the possibility that individual wasps might eventually open a shelter after repeated exposure or due to the passage of time, rather than as a result of experience with an unsheltered caterpillar. As was the case for 'pre-experience' trials, we recorded times and visits to each leaflet for each individual. We also noted whether any of the control foragers successfully killed a caterpillar.

Extraction times and methods for experienced wasps

After an experienced forager had subsequently extracted a sheltered larva on her own, we offered her additional sheltered *E. clarus* larvae to determine whether, having successfully removed a caterpillar once, she would do so again. To assess whether continued experience resulted in decreased larval extraction time, we normalized subsequent opening times to the time taken to extract the first caterpillar for each individual that opened at least three shelters. We used a repeated measures ANOVA to determine whether subsequent shelter-opening times differed from the initial time. We also recorded and categorized the methods used by all of the wasps to extract caterpillars, and then examined the methods used by each individual to determine whether they were consistent over time.

RESULTS

Pre-experience

We collected pre-experience data for 45 *P. fuscatus* and 13 *P. dominulus* foragers. Including all individuals, *P. fuscatus* foragers were observed for a total of 165.7 wasp-hours, and *P. dominulus* for 52.4 wasp-hours. We restricted our further analyses of time spent on leaflets and visit to leaflets to those individuals that had undergone at least three pre-experience trials, all of which took place with no more than 3 days intervening between subsequent trials. With these restrictions, our samples included 34 *P. fuscatus* foragers and nine *P. dominulus* foragers.

Time spent on leaflets

Foragers of both species spent about three times more of their total leaf time on leaflet C (including the shelter) than they did on either of the other two leaflets (repeated measures ANOVA: *P. fuscatus*: $F_{2,32} = 58.99$, $P < 0.001$; *P. dominulus*: $F_{2,7} = 30.87$, $P < 0.001$; Fig. 3). For both species, time spent on leaflet C (including the shelter) differed significantly from that on leaflets A and B (*P. fuscatus*: C versus A: $t_{33} = 9.35$, $P < 0.001$; C versus B: $t_{33} = 10.96$, $P < 0.001$; *P. dominulus*: C versus A: $t_8 = 4.49$, $P < 0.002$; C versus B: $t_8 = 7.08$, $P < 0.001$). The time spent on leaflets A and B did not differ for either species (*P. fuscatus*: $t_{33} = -0.71$, NS; *P. dominulus*: $t_8 = 0.08$, NS).

Visits to leaflets

The number of visits to leaflets followed the same pattern as did time spent on leaflets: *P. fuscatus* wasps visited the C leaflet about three times more often than they did the A and B leaflets (Fig. 4). Following a significant repeated measures ANOVA ($F_{2,32} = 83.52$, $P < 0.001$), we determined that the mean percentage of visits to leaflet C differed significantly from that to either the A or B leaflet (C versus A: $t_{33} = 11.49$, $P < 0.001$; C versus B: $t_{33} = 13.12$, $P < 0.001$), whereas the mean percentage of visits to A and B did not differ ($t_{33} = -0.55$, NS). We did not collect visit data for *P. dominulus*.

Rate of spontaneous shelter opening in pre-experience trials

As is clear from the above results, foragers of both species were 'interested' in the larval shelters. However, the vast majority of wasps did not open the shelters, even after extensive exposure (at least 3 h per wasp, for a total of > 165 wasp-hours for *P. fuscatus* and > 50 wasp-hours for *P. dominulus*). Only four of 45 (8.9%) *P. fuscatus* foragers were 'spontaneous learners'; that is, they extracted a caterpillar from its shelter during the course of their initial pre-experience period. None of the 13 *P. dominulus* foragers learned spontaneously (Fig. 5a, b).

Experience

A total of 25 *P. fuscatus* wasps (16 context, 9 noncontext) were given experience with an unsheltered caterpillar and participated in at least one postexperience trial. Seven *P. dominulus* foragers were given context experience and participated in at least one postexperience trial.

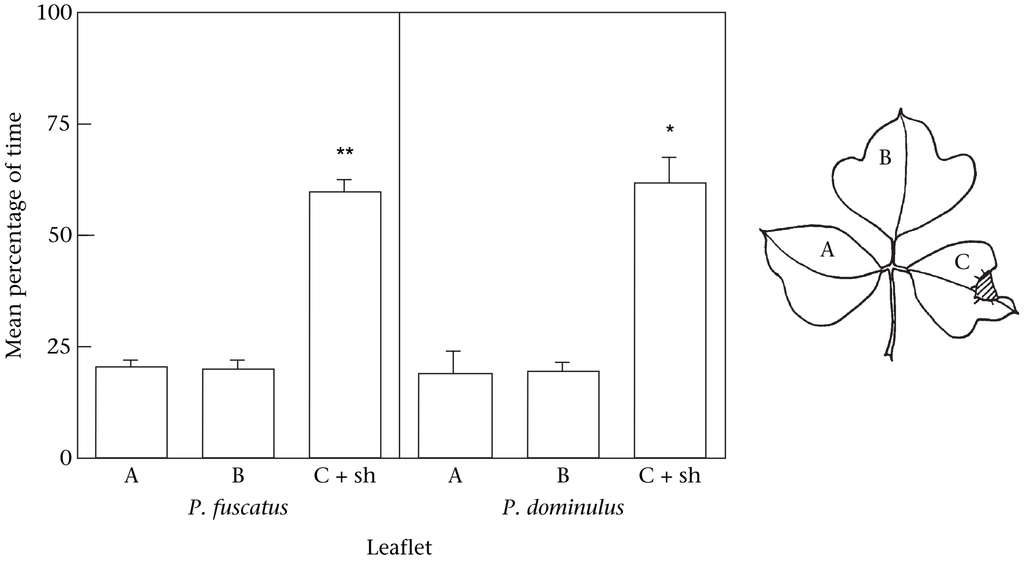

Figure 3. The mean percentage of time that naïve *Polistes fuscatus* ($N=34$) and *P. dominulus* ($N=9$) foragers spent on a leaflet on which an *E. clarus* larva had built a shelter (C + sh) and on two undamaged leaflets (A, B). $^*P < 0.01$; $^{**}P < 0.001$.

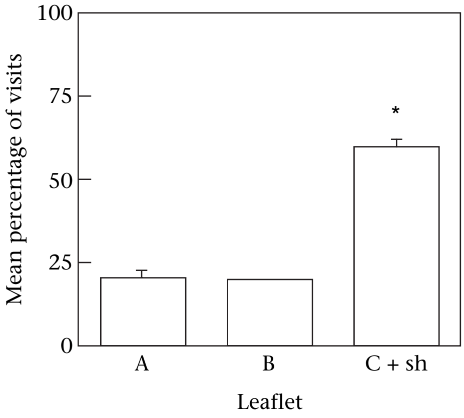

Figure 4. The mean percentage of visits by naïve *Polistes fuscatus* foragers ($N = 34$) to leaflets A, B and C + sh (as defined in Fig. 3). $^*P < 0.001$.

Postexperience

Controls

Fifteen *P. fuscatus* and seven *P. dominulus* wasps comprised the control population. Each wasp, in addition to her initial three pre-experience trials, was exposed to a sheltered caterpillar for an additional 2.8 ± 0.54 h ($\bar{X} \pm$ SE; range 1–8.2 h) over 3.7 ± 0.60 trials (range 2–9 trials) for *P. fuscatus*, and an additional 2.7 ± 0.83 h (range 1–6 h) over 2.3 ± 0.47 trials (range 1–4 trials) for *P. dominulus*. None of these wasps extracted or killed a caterpillar during their continued exposure to sheltered larvae (Fig. 5a, b).

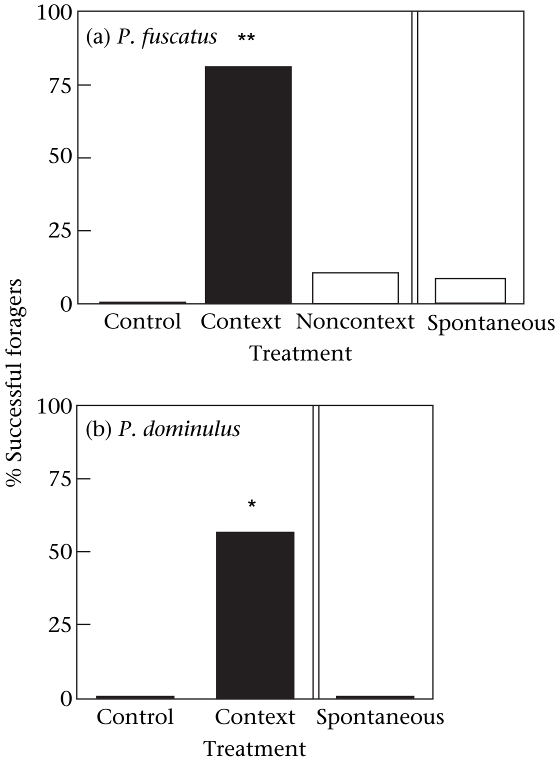

Figure 5. (a) The percentage of *P. fuscatus* foragers that successfully opened an *E. clarus* shelter during the pre-experience (spontaneous, $N = 45$) and postexperience phases (control, $N = 15$; context, $N = 15$; noncontext, $N = 9$). (b) The percentage of *P. dominulus* foragers that successfully opened an *E. clarus* shelter during the pre-experience (spontaneous, $N = 13$) and postexperience (control, $N = 7$; context, $N = 7$) phases. *Polistes dominulus* wasps were not given noncontext experience. $^*P < 0.05$; $^{**}P < 0.001$.

Context experiences

Thirteen of the 16 (81.3%) *P. fuscatus* individuals given context experience subsequently killed and removed a caterpillar from its shelter: 11 did so after a single experience and two did so following additional context experience (Fig. 5a). Four of the seven *P. dominulus* foragers (57.1%) given context experience subsequently opened *E. clarus* shelters and killed the resident caterpillars (Fig. 5b).

Noncontext experiences

Only one of the nine *P. fuscatus* foragers that was given ‘noncontext’ experience subsequently opened a shelter and killed the caterpillar (Fig. 5a).

Statistical results

Control, context-experienced and noncontext-experienced *P. fuscatus* wasps differed significantly in their ability to extract and kill sheltered caterpillars (2 × 3 chi-square test of independence: $\chi^2_2 = 25.39, P < 0.001$). Wasps given context experience were significantly more successful at opening shelters than were either control wasps or noncontext-experienced foragers (context versus control: $\chi^2_1 = 20.99$, $P < 0.001$; context versus noncontext: $\chi^2_1 = 11.5$, $P < 0.001$). Noncontext foragers did not differ from either control or spontaneous learners in number of caterpillars killed (noncontext versus control: $\chi^2_1 = 1.74$, NS; noncontext versus spontaneous: $\chi^2_1 = 0.046$, NS).

Repeat success

All 20 *P. fuscatus* foragers that successfully opened shelters (including those that did so spontaneously or following a single experience or re-experience) were presented with additional shelters. Fourteen of these wasps (70%) opened at least one additional shelter. Successful *P. dominulus* foragers were not offered additional shelters.

Extraction times

Of the 20 *P. fuscatus* foragers that opened one or more shelters ($\bar{X} \pm$ SE = 4.55 ± 0.83 shelters/wasp; range 1–13 shelters), nine wasps opened at least five shelters ($\bar{X} \pm$ SE = 8.11 ± 1.07 shelters; range 5–13). For this group of nine, we analysed any changes in time to kill or remove a caterpillar from its shelter over time (Fig. 6). We found no significant trend in the extraction times for *P. fuscatus* with increased trial number (repeated measures ANOVA: $F_{4,5} = 0.61$, NS).

Extraction methods

We observed 19 *P. fuscatus* wasps opening 87 shelters, and identified two general methods by which they did so. Wasps using a ‘caterpillar-focused’ strategy inserted their head into the shelter (or in some cases crawled into the

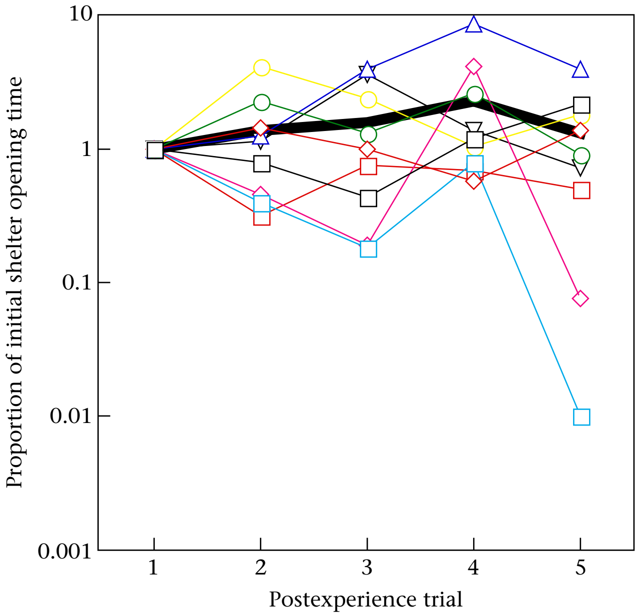

Figure 6. Extraction times for individual wasps, normalized to the time that it took each forager to open its first shelter. Each line represents an individual forager ($N = 9$ wasps); the dark black line shows the mean.

shelter) and pulled the caterpillar out, usually leaving the shelter intact. Wasps using a 'shelter-focused' strategy, on the other hand, either chewed through the shelter, cut the guy-wires that held the leaf flap to the leaf surface, or climbed atop the shelter and pulled back the leaf flap to rip the shelter open. The exposed caterpillar was then killed. Most foragers used a combination of the two strategies, although they tended to favour the 'caterpillar-focused' approach. Sixty-two shelters (71%) were opened by 17 of the wasps using a 'caterpillar-focused' strategy, whereas the remaining 25 shelters (29%) were opened by 12 of the wasps using a 'shelter-focused' strategy.

To examine the extraction methods used by an individual forager over time, we restricted our analysis to 12 foragers, each of which opened at least three shelters ($\overline{X} \pm \text{SE} = 6.5 \pm 0.97$ shelters/wasp; range 3–13 shelters/wasp). Eighty-three per cent (10 of 12) of these wasps used more than one method to open multiple shelters, whereas two of the 12 wasps used the same method 100% of time. Nine foragers favoured the caterpillar-focused method and two favoured the shelter-focused strategy, and one used both methods in equal proportions. For a given wasp, we found no evidence that one method was faster than another.

DISCUSSION

Many studies of learning in invertebrate predators have demonstrated an incremental improvement in the predator's performance. With experience, for example, spiders are faster at orienting to fruit flies (Morse 2000), shore-crabs require fewer attacks to kill mussels and dogwhelks (Cunningham & Hughes 1984), and mobile predatory crabs can opportunistically exploit common prey species (Micheli 1997). Such improvements may translate into a fitness advantage, as a result of increased rate of prey capture (Morse 2000), reduced exposure to environmental hazards (Rovero et al. 1999), or increased diet breadth (Micheli 1997). In this study we describe a dramatic example of learning, in which naïve wasps, often after only one experience, are able to overcome the defences of a previously inaccessible prey type. Learning to access a potentially abundant food source could provide an important fitness advantage for these generalist predators when alternative prey items are scarce.

Consistent with our original observations (Jones et al. 2002), we found that the vast majority (>90% for *P. fuscatus*; 100% for *P. dominulus*) of naïve wasps did not open larval leaf shelters in the pre-experience period, despite showing considerable interest in the shelters. At least two of the shelters that were opened in this period were loosely constructed; that is, there was a large gap in the region of the notch, or the flap was not securely silked down to the leaflet surface. The effect of an experience with an unsheltered larva depended in large part on the context in which it was encountered: 11 of 16 wasps that killed an exposed caterpillar on an open shelter (context experience) successfully opened shelters after a single encounter and two more did so after additional encounters. In contrast, only one of the nine wasps that killed a caterpillar on a petri dish or nonhost leaf (noncontext experience) subsequently opened a shelter, and none did so after additional noncontext experiences. The success rate of noncontext wasps did not differ significantly from that of spontaneous learners; nor did it differ from that of the control wasps, none of which opened leaf shelters.

What accounts for the difference in performance between groups? If, as above, we define learning as a modification of behaviour as a result of experience (Papaj & Prokopy 1989; Cunningham et al. 1998; Morse 2000), it is clear that the context-experienced wasps are learning something that the noncontext wasps are not, but what exactly are they learning? They are not learning to open the shelter per se, because we opened the shelter prior to their experience. The lack of learning in the noncontext group suggests that the wasps are not able to associate the odour of the caterpillar with its taste. Results of another experiment, in which we found that wasps did not differentiate between two intact shelters, one containing a caterpillar and the other unoccupied, lend further support to the idea that the wasps are not learning to recognize the odour of the caterpillar itself (M. R. Weiss, M. Conrad, E. Wilson & M. Brooks, unpublished data). Nor can we attribute the postexperience foraging success of the context-experienced wasps to an increased motivation to find a larva as a result of having tasted one, because again, the noncontext wasps shared this experience but did not learn. Finally, because the control wasps were not successful at opening shelters, we can rule out the possibility that the context group's learning came about as a result of repeated exposure to a sheltered caterpillar or the passage of time.

Predatory wasps are known to respond to visual and olfactory cues related to the presence of potential prey; these include leaf damage, regurgitate and other host-associated cues (Cornelius 1993; Raveret Richter 2000). We have found that *P. fuscatus* responds to the presence of silk, and to caterpillar-produced leaf damage (M. R. Weiss, M. Conrad, E. Wilson, M. Brooks, & J. Crawford, unpublished data). Thus, it seems likely that the context-experienced wasps learn to associate the taste of the caterpillar with shelter-related cues, such as the presence of silk or leaf damage. Such an association could involve olfactory cues, visual cues, or both; however, because the necessary experience takes place on an open shelter and the wasp subsequently opens a visually dissimilar closed shelter, olfactory cues may be more pertinent.

Might observational learning occur in predatory wasps, such that a naïve wasp could learn to open a shelter by watching an experienced forager do so? Although we did not test this possibility explicitly, we saw no evidence to suggest that observational learning was taking place, despite plausible opportunities for its occurrence. On a number of occasions naïve wasps were present on adjacent leaflets or leaflet C itself while an experienced forager was opening a shelter, so that the naïve wasp could presumably observe the forager's actions. However, we never saw an inexperienced 'observer' wasp subsequently open a shelter. Furthermore, because *Polistes* wasps from a given nest forage independently of one another, it seems unlikely that observational learning would routinely take place in the field.

If experience with a caterpillar in the context of its leaf shelter is necessary to enable the wasps to open shelters encountered subsequently, how then do wasps in the field become 'experienced'? We envision several ways in which this might happen. First, a foraging wasp could encounter an exposed caterpillar when the caterpillar was feeding or building a new shelter. Larvae leave their shelters to feed nearby for about 4 min/h, throughout the day (Lind et al. 2001). Larvae also abandon their old shelters and construct new ones about five times over the course of their larval ontogeny, and on each occasion are exposed for 0.5–2 h (Lind et al. 2001). In either situation, if a wasp saw and attacked a caterpillar close to its shelter, she might develop an association between the shelter cues and the taste of the larva. Second, if a forager encountered a loosely constructed shelter, or one that had been partially opened by a previous forager, she might be able to see the caterpillar inside and attack it. Third, it has been shown that the presence of other wasps on a prey item may increase the chance of a forager perceiving or locating the prey (Raveret Richter 2000). Therefore, it is possible that a wasp might gain the requisite experience if she encounters another wasp (conspecific or not) killing or processing a caterpillar on or near its shelter, and attempts to steal a portion or all of the prey item. Fourth, a wasp might encounter the partial remains of a large caterpillar, killed and left behind by a previous forager (Akre et al. 1980; Raveret Richter 2000), and, if the carcass is close to a shelter, might thus develop the necessary associations. Finally, our results with *P. fuscatus* show that about 10% of the wasps are spontaneous learners, and are able to open shelters on their own without explicit exposure to an unsheltered caterpillar.

The above scenarios generally depend on a forager encountering a caterpillar under a particular set of circumstances. Thus, the higher the density of caterpillars in the field, the more likely it is that a population of wasps will learn to take them as prey either through more frequent encounters with larvae or with other wasp foragers. Field densities of *E. clarus* larvae vary tremendously, depending on a number of factors, including spatial distribution of host plants, plant architecture and time in the season (M. R. Weiss, personal observation); thus rates of wasp encounter with larvae and opportunities for learning will also be quite variable.

Many invertebrate predators improve at locating or catching prey with increased experience (Raveret Richter & Jeanne 1985; Daniel & Bayer 1987; Rovero et al. 1999; Watanabe 1999). We were somewhat surprised, therefore, to see that larval extraction times for an individual wasp did not decrease with increased experience. Geitzenauer (1993) found a similar lack of improvement in handling times with increased experience in her study of *P. arizonensis* wasps foraging on caterpillars, and suggested that larval weight and volume of gut contents were important variables. Morse (2000) reported that although spiderlings oriented more quickly to their prey with increased experience, handling times did not decrease. The significant amount of 'noise' in our system, including variation in shelter style and tightness of construction, behaviour of the caterpillar in response to an attack, and motivation of the forager, could all affect extraction times, and might override any effect of forager experience. Alternatively, it is possible that experience with five caterpillars is not sufficient to yield a decrease in handling time, and that a decrease might be apparent following more exposures.

Lima has recently argued that predator behaviour has been 'left out' of studies of behavioural predator–prey interactions: 'Historically, we have been so focused on prey behavior that we … became comfortable with treating predators as unresponsive "black boxes" rather than participants in a behavioral interaction' (Lima 2002, page 70). Our results demonstrate that the experience level of predators can have a profound influence on their behaviour, and thus on the outcome of predator–prey interactions. Just as a particular type of defence may not provide equivalent protection against the range of generalist predators that occurs in natural systems (Olmstead & Denno 1993; Rayor 1996), it may not provide equal protection against predators with different levels of experience (Jones et al. 2002). In our system, leaf shelters provide an effective defence against naïve wasps, but are ineffective against the majority of context-experienced foragers. If experienced predators learn to recognize and cue in on visually obvious shelters, these defensive structures may even become liabilities for their inhabitants (Steiner 1984; Raveret Richter 1988; Jones et al. 2002). Consideration of the learning ability and experiential status of the predator will allow us to predict the outcome of predator–prey interactions more accurately.

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References

Akre, R. D. 1982. Social wasps. In: *Social Insects.* Vol. 4 (Ed. by H. Hermann), pp. 1–105. New York: Academic Press.

Akre, R. D., Greene, A., MacDonald, J. F., Landolt, P. J. & Davis, H. G. 1980. *Yellowjackets of American North of Mexico.* Washington, D.C.: U.S. Department of Agriculture.

Allen, T. J. 1997. *The Butterflies of West Virginia and their Caterpillars.* Pittsburgh, Pennsylvania: University of Pittsburgh Press.

Berenbaum, M. R. & Miliczky, E. 1984. Mantids and milkweed bugs: efficacy of aposematic coloration against invertebrate predators. *American Midland Naturalist*, **111**, 64–68.

Blois, C. & Cloarec, A. 1985. Influence of experience on prey selection by *Anax imperator* larvae. *Zeitschrift für Tierpsychologie-Journal of Comparative Ethology*, **66**, 303–312.

Clark, A. H. & Clark, L. F. 1951. *The Butterflies of Virginia.* Washington, D.C.: Smithsonian Institution Press.

Collett, T. S. 1995. Making learning easy: the acquisition of visual information during the orientation flights of social wasps. *Journal of Comparative Physiology A*, **177**, 737–747.

Cornelius, M. L. 1993. Influence of caterpillar feeding damage on the foraging behavior of the paper wasp, *Mischocyttarus flavitarsis* (Hymemoptera: Vespidae). *Journal of Insect Behavior*, **6**, 771–781.

Cunningham, J. P., West, S. A. & Wright, D. J. 1998. Learning in the nectar foraging behaviour of *Helicoverpa armigera*. *Ecological Entomology*, **23**, 363–369.

Cunningham, P. N. & Hughes, R. N. 1984. Learning of predatory skills by shorecrabs *Carcinus maenas* feeding on mussels and dogwhelks. *Marine Ecology Progress Series*, **16**, 21–26.

Damman, H. 1987. Leaf quality and enemy avoidance by the larvae of a pyralid moth. *Ecology*, **68**, 88–97.

Daniel, P. C. & Bayer, R. C. 1987. Development of chemically mediated prey-search response in postlarval lobsters (*Homarus americanus*) through feeding experience. *Journal of Chemical Ecology*, **13**, 1217–1233.

Dejean, A. 1988. Memory effect on predatory behaviour of *Odontomachus troglodytes* (Formicidae: Ponerinae). *Behaviour*, **107**, 131–137.

Eubanks, M. D., Nesci, K. A., Petersen, M. K., Zhiwei, L. & Sanches, H. B. 1997. The exploitation of an ant-defended host plant by a shelter-building herbivore. *Oecologia*, **109**, 454–460.

Gamboa, G. J., Greig, E. I. & Thom, M. C. 2002. The comparative biology of two sympatric paper wasps, the native *Polistes fuscatus* and the invasive *Polistes dominulus* (Hymenoptera, Vespidae). *Insectes Sociaux*, **49**, 45–49.

Geitzenauer, H. L. 1993. Tritrophic interactions: effects of caterpillar host plants on predatory paper wasps. M.S. thesis, University of Arizona.

Hughes, R. N. & Dunkin, S. D. 1984. Effect of dietary history on selection of prey, and foraging behavior among patches of prey, by the dogwhelk, *Nucella lapillus* (L.). *Journal of Experimental Marine Biology and Ecology*, **79**, 159–172.

Hughes, R. N. & O'Brien, N. 2001. Shore crabs are able to transfer learned handling skills to novel prey. *Animal Behaviour*, **61**, 711–714.

Jones, M. T., Castellanos, I. & Weiss, M. R. 2002. Do leaf shelters always protect caterpillars from invertebrate predators? *Ecological Entomology*, **27**, 753–757.

Karsai, I. & Hunt, J. H. 2002. Food quantity affects traits of offspring in the paper wasp *Polistes metricus* (Hymenoptera: Vespidae). *Environmental Entomology*, **31**, 99–106.

Krombein, K. V., Hurd, P. D., Smith, D. R. & Burks, B. D. 1979. *Catalog of Hymenoptera in America North of Mexico.* Washington, D.C.: Smithsonian Institution Press.

Lima, S. L. 2002. Putting predators back into behavioral predator–prey interactions. *Trends in Ecology and Evolution*, **17**, 70–75.

Lind, E. M., Jones, M. T., Long, J. D. & Weiss, M. R. 2001. Ontogenetic changes in leaf shelter construction by larvae of *Epargyreus clarus* (Hesperiidae), the silver-spotted skipper. *Journal of the Lepidopterists' Society*, **54**, 77–82.

Loeffler, C. C. 1996. Caterpillar leaf folding as a defense against predation and dislodgement: staged encounters using *Dichomeris* (Gelechiidae) larvae on goldenrods. *Journal of the Lepidopterists' Society*, **50**, 245–260.

McPheron, L. J. 1996. The role of learning in the foraging behavior of the paper wasp *Mischocyttarus flavitarsis*. Ph.D. thesis, University of California, Berkeley.

Micheli, F. 1997. Effects of experience on crab foraging in a mobile and sedentary species. *Animal Behaviour*, **53**, 1149–1159.

Michener, C. D. & Michener, M. H. 1951. *American Social Insects.* New York: Van Nostrand Rheinhold.

Morse, D. H. 2000. The effect of experience on the hunting success of newly emerged spiderlings. *Animal Behaviour*, **60**, 827–835.

O'Donnell, S. & Jeanne, R. L. 1992. Forager success increases with experience in *Polybia occidentalis* (Hymenoptera: Vespidae). *Insectes Sociaux*, **39**, 451–454.

Olmstead, K. L. & Denno, R. F. 1993. Effectiveness of tortoise beetle larval shields against different predator species. *Ecology*, **74**, 1394–1405.

Papaj, D. R. & Prokopy, R. J. 1989. Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology*, **34**, 315–350.

Pasteels, J. M. & Gregoire, J. C. 1984. Selective predation on chemically defended chrysomelid larvae: a conditioning process. *Journal of Chemical Ecology*, **10**, 1693–1700.

Persons, M. H. & Rypstra, A. L. 2000. Preference for chemical cues associated with recent prey in the wolf spider *Hogna helluo* (Araneae: Lycosidae). *Ethology*, **106**, 27–35.

Raveret Richter, M. 1988. Prey hunting and interactions among social wasp (Hymenoptera: Vespidae) foragers and responses of caterpillars to hunting wasps. Ph.D. thesis, Cornell University.

Raveret Richter, M. 2000. Social wasp (Hymenoptera: Vespidae) foraging behavior. *Annual Review of Entomology*, **45**, 121–150.

Raveret Richter, M. & Jeanne, R. L. 1985. Predatory behavior of *Polybia sericea* (Oliver), a tropical social wasp (Hymenoptera: Vespidae). *Behavioral Ecology and Sociobiology*, **16**, 165–170.

Rayor, L. S. 1996. Attack strategies of predatory wasps (Hymenoptera: Pompilidae; Sphecidae) on colonial or web-building spiders (Araneidae: *Metepeira incrassata*). *Journal of the Kansas Entomological Society*, **69**, 67–75.

Rayor, L. S. & Munson, S. 2002. Larval feeding experience influences adult predator acceptance of chemically defended prey. *Entomologia Experimentalis et Applicata*, **104**, 193–201.

Rovero, F., Hughes, R. N. & Chelazzi, G. 1999. Effect of experience on predatory behaviour of dogwhelks. *Animal Behaviour*, **57**, 1241–1249.

Scott, J. A. 1986. *The butterflies of North America; a Natural History and Field Guide*. Stanford, California: Stanford University Press.

Shafir, S. 1996. Color discrimination conditioning of a wasp, *Polybia occidentalis* (Hymenoptera: Vespidae). *Biotropica*, **28,** 243–251.

Steiner, A. L. 1984. Observations on the possible use of habitat cues and token stimuli by caterpillar-hunting wasps: *Euodynerus foraminatus* (Hymenoptera, Eumenidae). *Quaestiones Entomologicae*, **20,** 25–33.

Takagi, M., Hirose, Y. & Yamasaki, M. 1980. Prey-location learning in *Polistes jadwigae* Dalla Torre (Hymenoptera, Vespidae), field experiments on orientation. *Kontyû*, **48,** 53–58.

Turillazzi, S. & West-Eberhard, M.J. (Eds) 1996. *Natural History and Evolution of Paper-Wasps*. New York: Oxford University Press.

Watanabe, T. 1999. Web tuning of an orb-web spider, *Octonoba sybotides*, regulates prey-catching behaviour. *Proceedings of the Royal Society of London, Series B*, **267**, 565–569.