Female Spider Wasps, *Anoplius splendens* Driesbach (Hymenoptera: Pompilidae), Learn to Associate the Odor of Host Feces With the Presence of the Host[1]

F. Punzo[2]

University of Tampa, Department of Biology, Box 5F, 401 West Kennedy Boulevard, Tampa, Florida 33606-1490 USA

J. Entomol. Sci. 41(3): 202-210 (July 2006)

Abstract Laboratory studies were conducted to determine whether or not adult female wasps, *Anoplius splendens* (Dreisbach), can learn a chemical stimulus (host feces) associated with host microhabitats. In a two-choice, static-air olfactometer, wasps previously exposed to hosts only in association with one stimulus (filter paper conditioned with odors from feces of the host spider, *Drassodes auriculoides* Barrows) exhibited a greater tendency to visit exclusively, and spend more time in, chambers containing a source of that stimulus as compared to control wasps exposed to paper with no host odors. The capacity for learning olfactory cues based on previous encounters with hosts should enhance the ability of wasps to accurately identify microhabitats where suitable hosts are most likely to be located.

Key Words *Anoplius*, host microhabitat selection, olfactory learning, Pompilidae

Parasitoid wasps frequently learn to use visual and/or olfactory (chemical) cues associated with hosts or their microhabitats to enhance their ability to locate these microhabitats in heterogeneous environments where suitable hosts are likely to be found (Turlings et al. 1993, Kaiser et al. 2003, Punzo 2005). Chemical cues may be associated with specific chemical components of the cuticle or feces of the host (Punzo 2000, London and Jeanne 2005), with substrates on which hosts are found (Karguelen and Cardé 1996), or with oviposition sites (Turlings 1990, Kaiser et al. 1995). Because host-associated cues may vary in space and time, they can be unpredictable. In view of this, it has been suggested that the ability to identify chemical cues based on previous experience (learning) would allow a wasp to focus on specific cues that may lead it to a suitable host in a more efficient manner (Ma et al. 1992, Punzo 1994a), thereby decreasing energy that would be expended in random searching activities (Roitberg et al. 1993, Punzo 1994b). In contrast, parasitoids that search for hosts in homogeneous (predictable) environments are faced with fewer foraging decisions, suggesting that they should rely more on innate responses to specific cues (Vet et al. 1991, Potting et al. 1997).

One of the most interesting examples of odor learning has been demonstrated in parasitic wasps that have the ability to associate microhabitats in which a host may be found with odor cues associated with host frass or feces (Kats and Dill 1998, Punzo

[1]Received 28 July 2005; accepted for publication 21 January 2006.

[2] Email (fpunzo@ut.edu).

and Ludwig 2005). In some species, the host is initially located on the basis of an innate ability of the parasitoid to recognize chemical sign stimuli released by the host (Punzo 2005). In others, parasitoids spend significantly more time searching at sites containing chemosensory cues of specific host species that have been learned through experience (Vet et al. 1995, Punzo 2000).

Anoplius splendens (Dreisbach) (Hymenoptera: Pompilidae: Pompilinae) is a parasitoid wasp that occurs throughout the northeastern regions of the United States, extending southward to Georgia (Evans and Yoshimoto 1962). Female wasps have been reported to hunt a variety of hosts from several families of spiders including Lycosidae, Amaurobiidae, Gnaphosidae, and Salticidae) (Kurczewski et al. 1987). No information exists on the possible role of olfactory learning in subsequent host selection or microhabitat preference by this wasp. In this study, I tested the hypothesis that previous encounters with chemical cues associated with the feces of a host spider (*Drassodes auriculoides* Barrows) would affect subsequent host microhabitat preference by *A. splendens.*

Materials and Methods

Insect rearing methods. All wasps and hosts used in this study were from laboratory stock cultures that had been started in 2001 from adults originally collected in Erie Co., PA. Wasps were collected in microhabitats consisting of dense stands of shrubs and tall grasses. Larvae of *A. splendens* were reared on a species of the lycosid spider, *Schizocosa ocreata* (Hentz), that is used as a host by this wasp at the collection site. Eggs sacs of *S. ocreata* were maintained at 70% RH and 22 ± 0.2°C and 70% RH in Percival Model 85A environmental chambers (Boone, IA). Spiderlings were maintained individually in plastic dishes (10 cm diam), provided with water ad libitum, and fed on a diet of fruitflies (*Drosophila melanogaster* F.) and crickets (*Acheta* spp.). Older immature and adult spiders were fed crickets, mealworms (*Tenebrio molitor* Howe), and American cockroach (*Periplaneta americana* F.) nymphs.

The naturally-occurring host spider, *Drassodes auriculoides* Barrows (Araneae: Gnaphosidae) also was reared in the laboratory from males and females collected in Erie Co., PA, during 2001 and 2002. They were maintained under conditions identical to those described previously for *S. ocreata.*

Newly-emerged adult female wasps, with no prior encounter experience with the host, were maintained individually in well-ventilated plastic containers (20 × 15 × 10 cm) provided with a substrate of small wood shavings and a small square wooden block (length: 4 cm) that wasps used as a perch site when not wandering over the floor of the container. Containers were housed in a climate-controlled room at 21-23°C and 60-70% RH, under a 10L:14D h photoperiod regimen. This photoperiod regimen was chosen based on the previous success rate of rearing this wasp from egg through adult, followed by successful mating, under laboratory conditions. One female of the host *S. ocreata* was placed in each wasp container, and allowed to remain there until it had been attacked by the wasp, paralyzed, and an egg deposited upon it. The paralyzed spider and egg were then removed and maintained in an environmental chamber at 22°C, 70% RH, and constant darkness. Females and males that emerged were placed in plastic cages (30 × 30 × 60 cm) and fed a diet of honey and sucrose water. Females were randomly chosen from this population to serve as subjects in these experiments. Females were maintained individually in

plastic containers as described above. The protocol previously described ensured that these females had no prior encounter experience with a host and were considered naive.

Experiment 1-pretest conditions. Immediately following eclosion, naïve female wasps were placed in pretest microhabitats for 1 wk prior to testing. All wasps were exposed to two microhabitats that differed in the types of chemical cues present. Microhabitats were established using 200-ml styrofoam cups (Parker Co., Akron, OH). A piece of filter paper (Fisher, S47571A, 5.5 cm diam, Chicago, IL) was taped to the inside of the lid on each cup. One microhabitat consisted of cups containing chemical cues of host spider feces. This was accomplished by using filter papers that had been placed for 1 wk in 900-ml glass bottles, each containing 4 g of feces obtained from cages housing *D. auriculoides.* Each 900-ml bottle contained a single piece of filter paper (5 cm diam). Filter papers conditioned in this way (feces-scented) were considered as containing cues associated with the microhabitats of the host under natural conditions. The other microhabitat consisted of cups containing untreated filter paper. Each cup (n = 100 for each microhabitat) contained one wasp.

A single female of D. *auriculoides* (host) was presented to each female wasp residing in a microhabitat cup according to the following experimental design. Group I female wasps were presented with a host spider only in cups containing the scent of host feces. Each Group II wasp was presented with a host in a cup with untreated filter paper. With respect to control groups, wasps in Group III were presented with a host in cups containing the odor of host feces and control paper. Group IV wasps were treated in a similar fashion with the exception that hosts were absent. This pretest procedure was conducted over a period of 1 wk. Fresh hosts and new cups were provided each day.

At the end of the final day of pretesting, all wasps were marked on the thorax using a series of dots affixed with yellow paint to identify their treatment group. They were then placed by treatment group in a 40 × 40 × 30 cm holding cage and provided with honey and water containing sucrose.

Olfactometer testing procedures. On day 8, each wasp was tested in a 2-choice, static-air olfactometer (Fig. 1) that has been used by previous investigators with other parasitoid species (Wardle and Borden 1989). The olfactometer consisted of a an opaque 150 × 25 mm disposable Petri dish (Wards Natural Science, Model 17W0737, Rochester, NY) that served as a central opaque release chamber (RC), and 2 smaller Petri dishes (100 × 20 cm; Wards, Model 18W7105) that served as stimulus chambers (SC1, SC2). The walls of the SCs were cut in such a way that each SC could be affixed to opposite sides of the RC. Two 35 × 30 mm holes were cut through the walls of the RC and attached SCs allowing female wasps to leave the RC and enter either of the SCs. The RC and SCs were provided with lids which allowed for the removal of test subjects and cleaning of chambers between trials. A small plastic tube, 5 cm in length and 2 cm in diam, was attached to a centrally-located 15 mm hole in the ceiling of the RC through which a wasp was introduced into the RC at the beginning of a trial.

All tests were conducted in a room without windows. A cool fluorescent light was placed directly above the olfactometer. The olfactometer was placed in a 30 × 30 × 20 cm wooden box lined with white paper (Fig. 1, W) to minimize any effect of external visual stimuli. One SC contained a piece of conditioned filter paper (feces-scented) that had been allowed to remain for 1 wk in sealed bottles containing feces of host spiders as described previously, whereas the other SC contained untreated filter

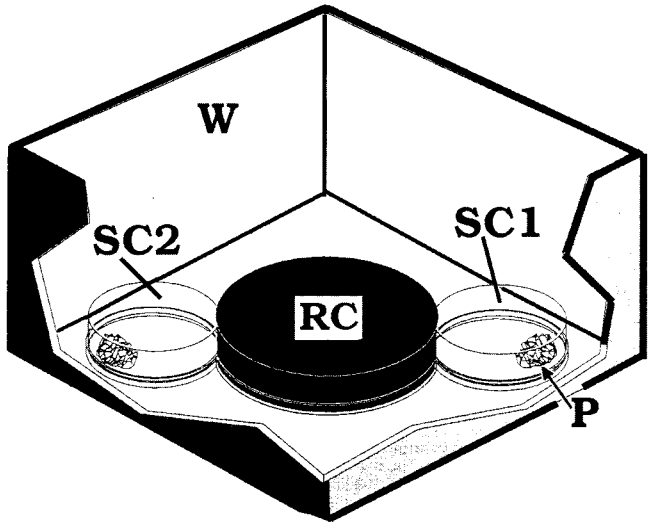

Fig. 1. Diagrammatic representation of the two-choice static air olfactometer used to test the responses of the wasp, *Anoplius splendens* to chemical stimuli. The olfactometer was placed in a 30 × 30 × 20 cm wooden box with white walls (W). RC = release chamber (150 × 25 mm Petri dish); SC1, SC2 = stimulus chambers (100 × 20 mm); W = wall; P = filter paper. An individual wasp was allowed to enter the RC through a centrally-located opaque tube (length: 5 cm; diam: 2 cm, not shown) affixed to an opening at the top of the RC. See text for details.

paper. Ten female wasps from each of the 4 groups were tested daily. At the start of a test, a wasp was removed from the holding cage in a black 4-dram shell vial (Fisher Scientific, S31644, Chicago, IL) and was released by inverting the vial over the tube leading into the RC of the olfactometer. Each subject was allowed to remain in the olfactometer for a period of 10 min from the time of entry. The following data were recorded for each test: whether the wasp entered either SC, and duration of time spent in each chamber. If a wasp did not leave the vial within 10 min, she was replaced with another individual of the same treatment group.

On each day of testing, wasps were tested in random order in blocks of 2 subjects, each consisting of one female from each group. Four identical olfactometers were used throughout these experiments. After each 10-min trial, all chambers were cleaned with a damp sponge and Sparkleen detergent (Fisher Scientific, S70110, Chicago, IL), allowed to dry, and fresh pieces of filter paper added. To avoid any position effect, the positions of the SC containing the feces-scented or untreated filter paper were reversed on each day of testing. An intertrial interval of 15 min was used throughout these experiments. Testing continued each day until 34 wasps from each group had been tested.

Experiment 2. Because of a concern that repeated exposure of group IV females could reduce their response to stimuli in general via habituation, in a follow-up ex-

periment, female wasps eclosing over a 24-h period were divided into two groups. Group IV2 females were exposed to an identical pretest regimen as group IV females in the previous experiments. Group V females were not presented with hosts or microhabitat cups. Following a 1-wk pretest period, 24 wasps from each treatment group were tested under identical procedures described above.

Statistical analyses. All statistical tests followed procedures outlined by Sokal and Rohlf (1995). In experiment 1, subjects were classified according to whether they had entered only one of the SCs, both chambers, or neither chamber. The number of subjects in each of these categories was compared using a 4 × 4 Chi Square contingency test. For significant Chi Square values ($P < 0.05$), simultaneous 95% confidence intervals were determined for differences between group I and groups II–IV only with respect to the proportion of wasps entering the SC containing feces-scented filter paper, and between group II and groups I, III, and IV with respect to the number of wasps entering only the SC with untreated paper. The mean proportion of total time spent in SCs that was spent in the feces-scented chamber was determined for all subjects in all groups. These means were compared using a Kruskal-Wallis test with multiple comparisons.

In experiment 2, data for groups IV2 and V were categorized on the basis of whether wasps entered either SC alone or both chambers, and then compared using a 2 × 3 Chi Square test. Mean proportions of total time spent in SCs that were spent in the feces-scented chamber by wasps in both groups were compared using a Mann-Whitney test.

Results

Almost all wasps entered stimulus chambers during testing (Table 1). In addition, wasps exhibited the capacity to learn chemical stimuli associated with host feces. Such stimuli could serve as cues and would be present in the microhabitat of the host.

Table 1. Percent of total time in olfactometer stimulus chambers (SCs) spent by various groups of females of *Anoplius splendens* in the SC containing filter paper conditioned with chemical cues associated with feces of the host spider, *Drassodes auriculoides*. During a pre-test period, wasps ere presented with hosts either in cups containing filter paper conditioned with host feces (group I), or in cups containing untreated filter paper (group II). Control groups consisted of wasps that were offered hosts in both cups (group III) or neither cup (group IV). Data expressed as means (±SE); N = 34 wasps/group. Means for percent of total time followed by different letters are significantly different, Kruskal-Wallis test, $P < 0.05$).

Group	Number of wasps entering stimulus chambers	Mean percent of total time
I	20	81.4a (6.9)
II	20	29.6b (3.1)
III	19	36.8b (4.2)
IV	19	34.3b (2.8)

In experiment 1, only group I wasps exposed to hosts exclusively in cups containing feces-scented filter paper showed a marked preference for this chemical cue (Tables 1, 2). The manner in which wasps entered the SCs also showed significant differences between groups ($P < 0.01$), with wasps from group I entering only the feces-scented SC significantly more times than wasps in Groups II–IV (Table 2). In contrast, group II females did not exclusively enter the SC containing untreated filter paper significantly more than wasps in other groups, except for group I (Table 2). Results also showed significant differences between groups with respect to the distribution of time spent in SCs, with group I wasps spending 58% of their time in the feces-scented chamber (Table 2). However, wasps in groups II–IV only spent between 29.6-36.8% of their time in this SC, and did not differ significantly from each other in the proportion of time spent between chambers.

In experiment 2, a 1-wk exposure to cups containing no hosts did not alter the responses of wasps to feces-scented versus untreated filter paper. Wasps from group IV2 and V did not differ significantly in the numbers of wasps entering either SC alone (21.2 and 19.8%, respectively) or both chambers (16.3 and 18.4%, respectively) ($P > 0.05$), or in the distribution of time spent between chamber containing feces-scented versus untreated filter paper (Mann-Whitney Test, $P > 0.05$).

Discussion

These results indicate that the preference exhibited by group I wasps toward the feces-scented filter paper in experiment 1 can most likely be attributed to the learning of chemical cues associated with host spiders because control wasps in groups III and IV did not show a similar preference. It should be pointed out that these control wasps were exposed to identical stimuli as those in group I except there was no specific pairing between hosts and the feces-scented cup. Extending the period of exposure to chemical stimuli without reward that was experienced by wasps in group IV did not alter their preference for feces-scented filter paper. This was supported by

Table 2. Percent of *A. slendens* from groups I-IV (N = 24/group) in experiment 1 that entered only the stimulus chamber (SC) containing filter paper conditioned with scent of host feces or untreated paper, both chambers, or neither chamber in a two-choice olfactometer. Values in rows followed by different letters are significantly different ($P < 0.05$; Chi Square with stimultaneous 95% confidence intervals for differences between proportions).

	Percentage of wasps in response category			
Chamber entered	Group I[+]	Group II	Group III	Group IV
Feces-scented	58a	22b	24b	22b
Untreated paper	12a	48b	38b	44b
Both chambers	21a	23a	27a	27a
Neither chamber	9a	7a	11a	7a

[+] Pre-test treatments: wasps presented with host spiders either in cups containing filter paper conditioned with host feces (Group I), or in cups containing untreated paper (Group II). Control groups consisted of wasps offered hosts in both cups (Group III) or neither cup (Group IV).

the results from experiment 2 showing a similarity of response between group IV2 and V wasps. This suggests that the preference exhibited by group I wasps for feces-scented cues is not an innate response.

The amount of time spent by group I wasps in the chamber containing chemical cues from host feces might be attributed to a response by these wasps to the specific odor associated with host feces. Wasps may have learned to recognize this odor when they were initially exposed to hosts in cups during pretesting procedures. It has been suggested that, depending on the species, parasitoid wasps can learn to recognize specific semiochemicals in the feces of suitable hosts upon initial contact. For example, the braconid wasp, *Cotesia marginiventris* Lawrence, (Turlings et al. 1993) was able to associate surrounding odors from hosts and host feces with the possible presence of hosts and subsequently used these odors as cues to enhance its searching behavior. In addition, it is well known that parasitoid wasps have the ability to recognize plant volatiles released as the result of damage to leaves caused by the feeding activities of phytophagous hosts, and to use these chemical cues to enhance the location of hosts (see review by Turlings et al. 1993, Vet et al. 1995).

In its natural environment, the ability of females of *A. splendens* to locate suitable arachnid hosts would be enhanced if they had the ability to respond to host-specific cues either via innate responses or through learning. *Drassodes auriculoides* or *S. ocreata,* known hosts of *A. splendens,* are members of families containing ground-hunting spiders that routinely deposit feces on the ground in areas (microhabitats) where they feed and seek shelter (Foelix 1996). The results of the present study show that *A. splendens* has the ability to respond to cues associated with host spider feces, which should enhance its host searching behavior. In addition, these experiments also show that for *A. splendens* this ability is most likely due to learning as opposed to the types of innate responses exhibited by many other species of parasitoid insects (Thorpe 1963, Vet et al. 1995). This type of learning, whereby an animal learns to associate certain odors (chemical stimuli) with an increased probability of encountering a prey (or predators), has been referred to as association learning by some investigators (Rosenheim 1993, Punzo 1996), and as olfactory conditioning by others (Thorpe 1943).

In conclusion, the learning of chemical cues associated with a host microhabitat should increase the ability of *A. splendens* to make decisions concerning the quality of certain patches and which patches to choose to maximize the probability of encountering a suitable host. This would take on added importance in microhabitats that contain dense stands of shrubs or taller grasses where visual cues might be obstructed, which represents precisely the types of habitats occupied by many populations of *A. splendens.*

Acknowledgments

The author thanks K. Lawrence, W. A. Gardner, and an anonymous reviewer for commenting on an earlier draft of the manuscript, and T. Ferraioli, L. Ludwig, and J. Bottrell for assistance in the collection of wasps and hosts in the field. Financial support for this research was provided by a Faculty Development Grant from the University of Tampa.

References Cited

Evans, H. E. and C. M. Yoshimoto. 1962. The ecology and nesting behavior of the Pompilidae (Hymenoptera) of the northeastern United States. Misc. Publs. Entomol. Soc. Amer. 3: 65-119.

Foelix, R. F. 1996. Biology of spiders. Oxford Univ. Press, NY.

Kaiser, L., C. Bartheye, V. Karguelen and M. H. Pham-Delegue. 1995. Odour conditioning of ovipositor probing in a parasitic wasp. Ethol. Ecol. Evol. 7: 245-255.

Kaiser, L., R. Perez-Maluf and M. H. Pham-Delegue. 2003. Dynamics of odour learning in *Leptopilina boulardi,* a hymenopteran parasitoid. Anim. Behav. 66: 1077-1084.

Karguelen, V. and R. T. Cardé. 1996. Reinforcement mechanisms of olfactory conditioning during parasitization by the parasitoid *Brachymeria intermedia* (Hymenoptera: Chalcididae). J. Insect Behav. 9: 947-960.

Kats, L. B. and L. Dill. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience 5: 361-394.

Kurczewski, F. E., E. J. Kurczewski and R. A. Norton. 1987. New prey records for species of Nearctic Pompilidae (Hymenoptera). J. Kans. Entomol. Soc. 60: 467-475.

London, K. B. and R. L. Jeanne. 2005. Wasps learn to recognize the odor of local ants. J. Kans. Entomol. Soc. 78: 134-141.

Ma, R. Z., P. D. Swedenborg and R. I. Jones. 1992. Host-seeking behavior of *Eriborus terebrans* (Hymenoptera: Ichneumonidae) toward the European corn borer and the role of chemical stimuli. Ann. Entomol. Soc. Am. 85: 72-79.

Potting, R. P. J., H. Otten and L. E. M. Vet. 1997. Absence of odour learning in the stemborer parasitoid *Cotesia flavipes.* Anim. Behav. 53: 1211-1223.

Punzo, F. 1994a. The biology of the spider wasp, *Pepsis thisbe* (Hymenoptera: Pompilidae) from Trans Pecos Texas. II. Temporal patterns of activity and hunting behavior with special reference to the effects of experience. Psyche (Stuttg.) 101: 243-256.

Punzo, F. 1994b. The biology of the spider wasp, *Pepsis thisbe* (Hymenoptera: Pompilidae) from Trans Pecos Texas. I. Adult morphometrics, larval development and the ontogeny of larval feeding. Psyche (Stuttg.) 101: 229-241.

Punzo, F. 1996. Localization of brain function and neurochemical events associated with learning in insects. Rec. Trends Comp. Biochem. Physiol. 2: 9-16.

Punzo, F. 2000. Desert arthropods: life history variations. Springer, Heidelberg.

Punzo, F. 2005. Studies on the natural history, ecology and behavior of *Pepsis cerberus* and *Pepsis mexicana* (Hymenoptera: Pompilidae) from Big Bend National Park, Texas. J. New York Entomol. Soc. 113: 84-95.

Punzo, F. and L. Ludwig. 2005. Behavioral responses of *Pepsis thisbe* (Hymenoptera: Pompilidae) to chemosensory cues associated with the host spiders. J. Insect Behav. 18: 757-766.

Roitberg, B. D., M. L. Reid and C. Li. 1993. Choosing hosts and mates: the value of Learning, Pp. 174-193. *In* D. R. Papaj and A. C. Lewis [eds.], Insect learning: ecological and evolutionary perspectives. Chapman and Hall, NY.

Rosenheim, J. A. 1993. Comparative and experimental approaches to understanding insect learning, Pp. 273-307. *In* D. R. Papaj and A. C. Lewis [eds.], Insect learning: ecological and evolutionary perspectives. Chapman and Hall, NY.

Sokal, R. F. and B. J. Rohlf. 1995. Biometry. Third Ed. W. H. Freeman, NY.

Thorpe, W. H. 1943. Types of learning in insects and other arthropods. Br. J. Psychol. 33: 220-234.

Thorpe, W. H. 1963. Learning and instinct in animals. Methuen, London.

Turlings, T. C. J. 1990. Semiochemically mediated host searching behavior of the endoparasitic wasp *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). Ph.D. Diss., Univ. of Florida, Gainesville.

Turlings, T. C. J., F. L. Wackers, L. E. M. Vet, W. J. Lewis and J. H. Tumlinson. 1993. Learning of host-finding cues by Hymenopterous parasitoids, Pp. 51-78. *In* D. R. Papaj and

A. C. Lewis [eds.], Insect learning: ecological and evolutionary perspectives. Chapman and Hall, NY.

Vet, L. E. M., F. L. Wackers and M. Dicke. 1991. How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. Neth. J. Sci. 41: 202-213.

Vet, L. E. M., W. J. Lewis and R. T. Cardé. 1995. Parasitoid foraging and learning, Pp. 65-104. *In* R. T. Cardé and W. J. Bell [eds.], Chemical ecology of insects. Second ed. Chapman and Hall, NY.

Wardle, A. R. and J. K. Borden. 1989. Learning of an olfactory stimulus associated with a host microhabitat by *Exeristes roborator*. Entomol. Exp. Appl. 52: 271-279.