DOI: 10.1111/j.1570-7458.2008.00743.x

MINI REVIEW

Development of *Microplitis croceipes* as a biological sensor

J. K. Tomberlin[1]*, G. C. Rains[2] & M. R. Sanford[1]

[1]*Department of Entomology, Texas A&M University, College Station, TX 77845-2475, USA, and* [2]*Department of Biological and Agricultural Engineering, University of Georgia, Tifton, GA 31783, USA*

Accepted: 2 May 2008

Key words: associative learning, Wasp Hound®, Hymenoptera, Braconidae, conditioning, medical diagnosis, forensics, food safety, national security, plant disease

Abstract

Classical conditioning, a form of associative learning, was first described in the vertebrate literature by Pavlov, but has since been documented for a wide variety of insects. Our knowledge of associative learning by insects began with Karl vonFrisch explaining communication among honeybees, *Apis mellifera* L. (Hymenoptera: Apidae). Since then, the honey bee has provided us with much of what we understand about associative learning in insects and how we relate the theories of learning in vertebrates to insects. Fruit flies, moths, and parasitic wasps are just a few examples of other insects that have been documented with the ability to learn. A novel direction in research on this topic attempts to harness the ability of insects to learn for the development of biological sensors. Parasitic wasps, especially *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), have been conditioned to detect the odors associated with explosives, food toxins, and cadavers. Honeybees and moths have also been associatively conditioned to several volatiles of interest in forensics and national security. In some cases, handheld devices have been developed to harness the insects and observe conditioned behavioral responses to air samples in an attempt to detect target volatiles. Current research on the development of biological sensors with insects is focusing on factors that influence the learning and memory ability of arthropods as well as potential mathematical techniques for improving the interpretation of the behavioral responses to conditioned stimuli. Chemical detection devices using arthropod-based sensing could be used in situations where trained canines cannot be used (such as toxic environments) or are unavailable, electronic devices are too expensive and/or not of sufficient sensitivity, and when conditioning to target chemicals must be done within minutes of detection. The purpose of this article is to provide a brief review of the development of *M. croceipes* as a model system for exploring associative learning for the development of biological sensors.

Harnessing nature for the development of biological sensors

Interactions between individuals involve receiving and interpreting information from the environment as well as from one another. Depending on the type of information, a variety of responses can be produced. In many cases, the ability to detect, recognize, and respond quickly to these stimuli is essential for survivorship. Delayed responses or incorrect responses to a stimulus can reduce the likelihood of acquiring a resource, such as food. When viewed at a greater ecological level, incorrect responses can be the difference between the life and death of an individual.

Significant research has focused on understanding how plants and animals receive information from their environment. These efforts are wide ranging, from behavioral to cellular responses to stimuli. Such studies provide insight to animal and plant ecology. In addition, such research

---

*Correspondence: Jeffery K. Tomberlin, Department of Entomology, Texas A&M University, College Station, TX 77845-2475, USA.
E-mail: jktomberlin@ag.tamu.edu

efforts could lead to the development of biological sensors (Habib, 2007). These sensors could potentially be based on cellular responses (Park et al., 2002) or behavioral responses (Rains et al., 2004) to external stimuli. Bleckmann et al. (2004) provide an excellent review of sensory systems at the cellular level and the potential for modeling these systems for the development of biological sensors. In this review, we discuss the process of associative learning in the parasitic wasp *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) and its use as a model for the development of a biological sensor.

A brief history of associative learning in arthropods

Learning appears to be a ubiquitous attribute of insects in at least some form. A greater understanding of learning in insects has substantial consequences for improving biological control programs (Prokopy & Lewis, 1993). In addition, harnessing insect learning and sensory abilities is leading to the development of biological sensors. There are two major difficulties that arise in the study of insect learning: (i) how does one define learning? and (ii) how does one go about investigating it?

Learning is not uniquely defined by all scientists. There are many definitions for animal-learning and its respective aspects in the literature (Kimble, 1961; Tully, 1984; Stephens, 1993), and it is a highly debated topic among behaviorists and ethologists (Gould, 1993; Vet et al., 1995). In his often-cited book on animal-learning and instinct, Thorpe (1963) describes learning as 'that process which manifests itself by adaptive changes in individual behavior as a result of experience.' As with any scientific definition there is debate, however, this particular definition captures the essence of learning.

Scientists developed some rudimentary definitions to aid investigations into learning mechanisms. Of those, the most widely accepted forms of learning in insects are habituation, sensitization, and associative learning (Alloway, 1972; Dethier, 1976; Papaj & Prokopy, 1989; Bernays, 1995). Habituation, which is a form of non-associative learning, involves the waning of a reflexive response to an unconditioned stimulus after repeated exposure and involves changes in neurotransmitter release at synaptic pathways (Lieberman, 1993; Bernays, 1995); as a result, the animal fails to respond to the stimulus over time with repeated exposure. Sensitization, another form of non-associative learning, is considered the opposite of habituation and involves heightened responsiveness to a stimulus with repeated exposure (Lieberman, 1993; Bernays, 1995).

Operant conditioning, or instrumental learning, is a form of associative learning described as rewarding positive or negative change in behavior over time (Skinner, 1953). For example, the repeated experience with a particular flower type by Lepidoptera and Hymenoptera that has a 'rewarding' nectar source decreases the amount of time the insect requires to extract the nectar from it on subsequent visits based on behavioral modifications to improve efficiency (Papaj & Prokopy, 1989). In other words, consequences – whether good or bad – determine if a behavior is maintained or not.

Classical conditioning, which is synonymous with Pavlovian or respondent conditioning, is another form of associative learning. This type of learning has the most potential for application toward the development of biological sensors. Associative learning is probably most widely known from the classical experiments of Pavlov in which he trained dogs by ringing a bell prior to offering them food (Pavlov, 1927). This type of learning involves the coupling of an unconditioned stimulus with a conditioned stimulus to elicit a response. In the case of Pavlov's work, this association would have been linking availability of food (unconditioned stimulus) with the ringing of a bell (conditioned stimulus) to induce salivation (unconditioned response) by a dog (Pavlov, 1927). Through a course of training by exposure of the conditioned stimulus with the unconditioned stimulus, the animal will display the unconditioned response with exposure to the conditioned stimulus (Gould, 1993). Thus, the dog salivated, now the conditioned response, with the ringing of the bell (conditioned stimulus).

Much of what we know about associative learning in insects comes from work with adult fruit flies in the genus *Drosophila* (Diptera: Drosophilidae) (Quinn et al., 1974), the honeybee *Apis mellifera* L. (Hymentoptera: Apidae) (von Frisch, 1956; Bitterman et al., 1983; Hammer & Menzel, 1995), and the tobacco hornworm *Manduca sexta* (L.) (Lepidoptera: Sphingidae) (Daly & Smith, 2000; Daly et al., 2001). A majority of the work published on associative learning in *Drosophila* is aversion learning in which the adult fly is conditioned to avoid an electrified area either in a maze or in a flight tunnel in conjunction with an odor (Quinn et al., 1974; Tully, 1984; Tully & Quinn, 1985). The availability of the *Drosophila* genome and the wide variety of mutants have made work on the genetic basis for behavior and learning in this genus possible (Tully, 1984; Tully & Quinn, 1985; Zars et al., 2000; Suh et al., 2004). In the honeybee, the proboscis extension reflex has been a valuable tool in examining associative learning and much of the work with bees has been at higher levels of the central nervous system (Meller & Davis, 1996). Work with honeybees has led to the discovery of the function of the antennal lobes and mushroom bodies in associative learning involving odors (Erber et al., 1980; Hammer & Menzel, 1998; Faber et al., 1999; Faber & Menzel, 2001; Sandoz et al., 2003; Wang et al., 2005). Recent work with *M. sexta* has led to simultaneous mapping of antennal lobe neural networks in the brain while training to odors (Daly et al., 2004). Studies in parasitoid wasp learning have explored the importance of both olfactory and visual cues in foraging and the associative learning capabilities of these wasps (Lewis & Tumlinson, 1988; Turlings et al., 1993; Wäckers et al., 2002).

A model system for examining associative learning by arthropods

Microplitis croceipes has served as a model for a number of studies examining the learning and foraging behavior of insects (Lewis & Martin, 1990; Takasu & Lewis, 1993, 1995, 1996). *Microplitis croceipes* is a parasitoid wasp commonly encountered throughout the temperate regions of North America. This wasp is a highly specialized endoparasitoid (Le & Takasu, 2005) with its primary hosts being *Helicoverpa zea* (F.) (Lepidoptera: Noctuidae) and *Heliothis virescens* (Boddie) (Lepidoptera: Noctuidae) (Lewis, 1970). It is categorized as a beneficial arthropod in row crops (Lewis, 1970), vegetable crops, and the cultivation of pine saplings (Herman & Davidson, 2000). In some cases, *M. croceipes* is known to parasitize *Helicoverpa armigera* (Hübner), which is a close relative of its primary hosts and a more serious agricultural pest in Asian countries (Herman & Davidson, 2000; Le & Takasu, 2005).

As previously mentioned, *M. croceipes* primarily relies on *H. zea* and *H. virescens* caterpillars as hosts for its offspring. Therefore, an intimate biochemical and physiological parasitoid–prey relationship has evolved (Le & Takasu, 2005). This relationship is tritrophic and involves not only the caterpillar and parasitoid, but also the plants fed on by the host larvae. Plants react to herbivore-inflicted damage by producing volatiles that attract parasitic arthropods (Turlings et al., 1993). Like other parasitoids, *M. croceipes* uses these olfactory as well as visual cues to locate host and food resources (Takasu & Lewis, 1993, 1995; Wäckers & Lewis, 1999) and it has been determined that the use of these informative cues is improved through associative learning (Drost et al., 1988). Learning cues associated with these resources can result in more efficient foraging in diverse habitats for these resources. Initially, it was thought that this ability was restricted to female *M. croceipes*. However, recent research has determined that male *M. croceipes* can learn and are as sensitive to select compounds as females (Takasu et al., 2007).

Methods employed in the laboratory to measure learning capabilities of *M. croceipes* are simplistic and easily implemented and are based on exposing individual wasps to target odors immediately before providing them with the unconditioned stimulus. Typically, wasps are starved 48 h prior to their use in studies examining associative learning in relation to food. In the case of Rains et al. (2004), individual wasps were conditioned to 1 mg of the target compound placed on a 2.5-cm diameter Whatman no. 1 filter paper (Fisher Scientific, Norcross, GA, USA). The filter paper treated with the target compound was placed in a 250-ml glass jar with a magnetic stirrer and covered with an 8 × 7 cm aluminum foil sheet sealed with a lid. The glass container then was placed on a magnetic stirrer set at 770 r.p.m. for 5 min in order to distribute the volatiles throughout the container. Seven 1-mm holes separated by a minimum of 2 mm then were placed in a circle near the center of the foil. Approximately 0.5 ml droplet of 33% sucrose solution, which served as a food source for the wasps, was placed in the center of the ringlet of holes. Individual wasps were then placed on the aluminium foil and allowed to feed for 10 s three times on the sucrose solution with approximately 3 min between sessions on the assumption that the wasps were exposed to the volatiles of the target compound emitting from the holes. Wasps were considered conditioned after the third feeding interval. Wasps at this point were held individually for 15 min in 5-ml glass vials before testing. This design, but without the sucrose solution, was used to test the behavioral responses of each conditioned wasp. Conditioned wasps were released in the center of the circle of holes from which an odorant would diffuse. The amount of time the wasps remained within a 1-cm radius of the holes searching (i.e., antennating around the holes) was recorded. Wasps responding for <10 s were recorded as negative responses. Longer responses were considered positive detections of the target odorant. This behavioral response is the primary response utilized by the Wasp Hound® (University of Georgia, Tifton, GA, USA) biological sensor, which will be discussed below, for the detection of target odors.

The same timing regiments also are used when working with conditioning wasps to cues in association with hosts (Olson et al., 2003). However, the conditioning methods are somewhat different. Female wasps are exposed to a target odor while antennating feces from diet-fed third-instar *H. zea* (Olson et al., 2003). Female *M. croceipes* that have successfully been conditioned to link separate volatiles for food or host will exhibit resource- (i.e., food- or host-) specific behavioral responses when these volatiles are subsequently encountered (Olson et al., 2003). For example, individuals conditioned to an odor in association with food will respond by antennating the surface on which they are resting and migrating toward the odor source when exposed to the target odor (Wäckers et al., 2002). Females conditioned in association with hosts will exhibit a coiling response, the behavior exhibited right before attacking their hosts when contact with the target odor is made (Olson et al., 2003). In contrast, naïve *M. croceipes* will not exhibit these behaviors when exposed to novel odors (Olson et al., 2003). Additionally, individuals can also successfully link an odor in association with successful oviposition in a host. And they were more likely to remember that odor 24 h later, indicating that ovipositor contact with the host can result in associative learning taking place (Takasu & Lewis, 2003).

The ability to detect and discriminate between volatiles learned in association with a resource and other odors of similar structure is highly evolved in this wasp. Conditioned female *M. croceipes* are able to discriminate, in flight tunnel tests, between isomers of six carbon alcohols based on position of the alcohol group (Meiners et al., 2002). However, the likelihood of distinguishing between closely related volatiles depends on chain length and the role functional groups on the molecules play in the natural history of *M. croceipes* (Meiners et al., 2002). Because behaviors exhibited by conditioned *M. croceipes* to target odors are predictable, efforts to harness this ability for the detection of volatiles of human importance have been made.

Development of a biological sensor

A handheld instrument called the Wasp Hound has been developed for the detection of volatile odors (Figure 1) using *M. croceipes* to sense the chemical volatiles (Rains et al., 2006). The Wasp Hound consists of a ventilated area, a mounted camera, a fixed light source, and a wasp chamber that can contain up to five wasps. Insects move freely within the wasp chamber (Figure 2) and the air sampling fan slowly draws outside air through the chamber. The mounted camera is used for observing insect behavior under consistent lighting (Figure 3).

Communication with the Wasp Hound is accomplished through the Visual Cortex® (University of Georgia) program. Image acquisition and analysis is performed using this software that is loaded on a laptop computer and connected to the Wasp Hound through a USB connector. The wasps inside the wasp chamber respond to odors through the conditioned behavioral responses discussed above. The chamber is well-ventilated, transparent, and illuminated with an LED, so the interior can be monitored by a web camera (Figure 3).

Data collection and analysis

The Wasp Hound is currently a positive/negative chemical odor detector. Detection of wasp response is made by taking five frames of video per second and detecting the food-searching behavior of the wasps aggregating around the exhaust port emitting the target odor. The searching behavior exhibited by the conditioned wasps is defined as area-restricted searching, which is a positive response.

The Wasp Hound uses an algorithm to transduce this behavior into quantifiable data (Figure 4). Wasps present around the exhaust port are converted into black pixels that are counted and compared to the total number of

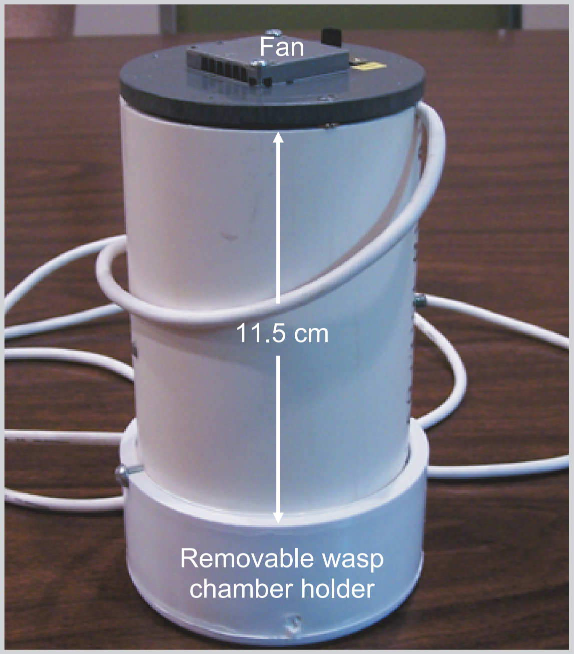

Figure 1 The Wasp Hound integrates computer imaging into a portable handheld device. The Wasp Hound provides consistent lighting, chamber placement, and air flow.

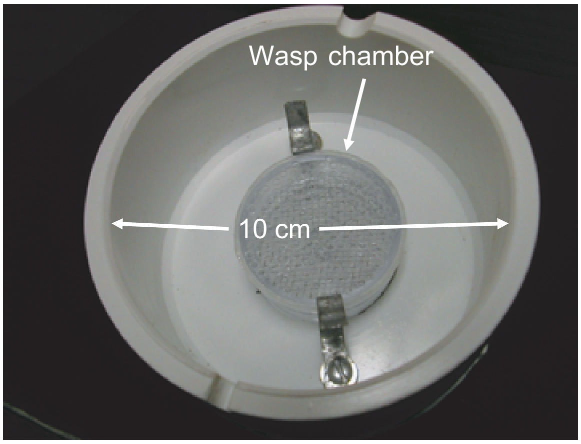

Figure 2 Wasp chamber holder when removed from Wasp Hound. Wasp chamber is shown with holding clips.

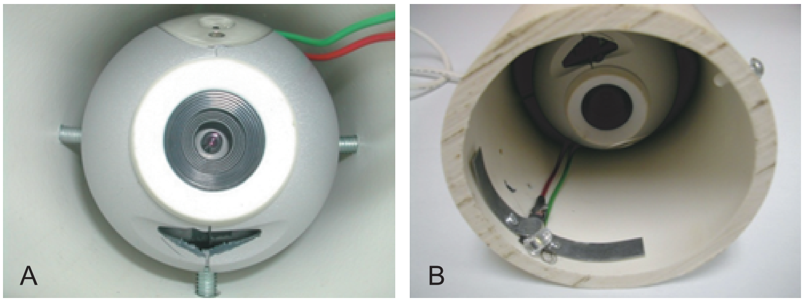

Figure 3 Mounting of camera and LED within Wasp Hound. (A) The web cam is suspended within the Wasp Hound 2.5 cm above the wasp chamber. (B) An LED is mounted within the Wasp Hound as the main source of illumination.

pixels available in each video frame. This time-variant information is then integrated to provide a cumulative response of the aggregating conditioned wasps around the exhaust port.

Two basic responses are depicted in Figure 4. Non-target odors result in negative responses, since they do not elicit the area-restricted searching behavior, and the wasps apparently move randomly within the chamber of the Wasp Hound. Conversely, positive responses result in the wasps aggregating in the vicinity of the exhaust port. Cumulative summaries of pixilated wasp activity around the exhaust port over time will result in either a significantly steep (positive due to wasp aggregation) or gentle (negative due to wasps moving randomly) slope of the curve response, which is then interpreted by the operator. These responses can be determined within 20–30 s of continuous sampling of a site for the target odor.

Furthermore, the Wasp Hound is designed for sampling sources with a headspace providing a continuous flow of the target odorant. If the odor is continuous, a constant slope will be depicted on the computer monitor as depicted in Figure 4. The slope will plateau during periods when the odor is intermittent. At this time, no data are available on the false-positive rates of the Wasp Hound.

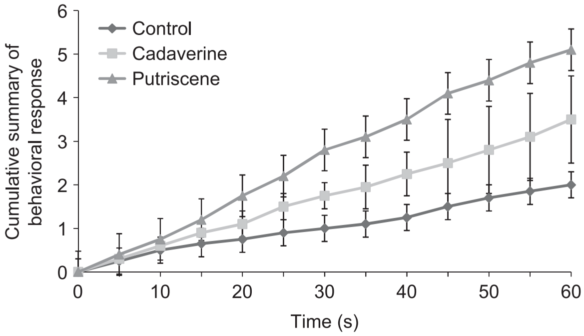

Figure 4 Cumulative response curves (mean; n = 6) with 90% confidence interval based on pixel imaging recorded from Wasp Hound when five *Microplitis croceipes* females were conditioned to putriscene and tested to putriscene, cadaverine, and a control (air).

In this response curve, wasps were conditioned to putriscene in association with food using methods previously described. They were then tested to the same dosage of putriscene, cadaverine, and a control of dimethyl chloride. The sloped lines are averages of six tests where the Wasp Hound sampled air from a 200-ml jar containing 10 μl of the pure substance on filter paper. Newly conditioned wasps were used in each test. The 90% confidence interval indicates that the Wasp Hound detected and differentiated putriscene from cadaverine and the control within 25 s.

Putriscene and cadaverine are odors typically produced by decomposing corpses. Studies examining succession and concentration of class compounds under controlled field conditions have been conducted (Vass et al., 2002, 2004). But, it is not clear what the concentrations of the specific odors are over time and what specific compounds are actually from the remains vs. the surrounding environment. Therefore, examining the ability of the Wasp Hound to detect known concentration of putriscene and cadaverine encountered from a buried decomposing corpse is not possible at this time.

Potential and pitfalls

The Wasp Hound has the potential to detect volatile compounds associated with medical diagnoses, forensic applications, food safety, and national security. It also has been used to detect chemicals associated with aflatoxin in corn (Rains et al., 2006). Tests with wasps have also shown their ability to detect explosives (Tomberlin et al., 2005) and plant disease (Rains et al., 2004).

Wasps are extremely sensitive detectors. In nature, they must navigate to their food and host resources within large and diverse environments. Wasp responses to target odors have been compared with a Cyranose 320 Electronic nose and were found to be almost 100 times more sensitive to low concentrations of fungal and plant odors (Rains et al., 2004). Wasps can also discriminate a target odor within a bouquet of other odors. This ability is another advantage over most electronic nose technologies that focus on pattern recognition of a specific odor bouquet. Masking of the odor causes the electronic device to miss the odor of interest.

Other portable biological sensors with arthropod components have been developed. An electroantennogram composed of antennae from *Drosophila melanogaster* Meigen, *H. virescens*, *H. zea*, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), and *M. croceipes* is able to detect and discriminate 20 volatile compounds under field conditions (Park et al., 2002). Additionally, a prototype sensor that monitors electromyographic signals of conditioned *M. sexta* adults to detect explosives has been developed (King et al., 2004). However, like the Wasp Hound, the detection thresholds of these devices to target compounds in the field are not known. Accordingly, another concern is the lack of field research examining the ability of these devices actually to detect odors from clandestine graves or explosives to determine their real-world practicality.

One of the most remarkable abilities that *M. croceipes* possesses is the ability to be conditioned to an odor in a very short period of time. A single wasp can be conditioned to an odor within 5 min. Consequently, wasps can be conditioned when needed and very quickly. This fast turnaround from training to use provides for multiple logistical possibilities when using wasps for different applications. Wasps can be conditioned in the laboratory and shipped to the location of the application, or they can be shipped as cocoons or adults to the location and conditioned on the spot.

On the other hand, biological sensors based on insect models that have been developed are still in the laboratory phase. No field studies examining the sensitivity and reliability of these systems in 'real-word' scenarios have been conducted. This hurdle applies to the Wasp Hound. However, at the time of preparing this article, research is being initiated to examine the ability of the Wasp Hound to distinguish between soil samples from sites with and without buried decomposing vertebrate remains.

Wasp Hound sensing poses unique challenges that hinder the adoption into many applications. Each wasp detects odors based on the drive to find food. Once they have been exposed to that odor 2–3 times without food reinforcement, their sensing of the odor diminishes rapidly. This also makes it difficult to devise a system for continuous sampling when a high rate of positive responses is expected. In that case, wasp chambers need to be replaced often, with a new set of conditioned wasps during each replacement. These hurdles are not unique to *M. croceipes*, but can be expected with the use of other arthropods as biological sensors. Wasps and insects in general are also susceptible to changing environmental conditions. Hot and cold conditions can kill wasps and/or possibly affect their behavioral response. The effects of changing environmental conditions on longevity and odor sensitivity have not yet been researched. Development of a reinforcement protocol and an environmentally controlled wasp chamber could alleviate these issues.

Future prospects

Experimental design and parameters utilized to conduct learning experiments significantly influence results generated (Holland, 2004). Initial studies examining the conditioned response of female *M. croceipes* to target volatiles defined a basic methods platform for conditioning and testing the wasps (Takasu & Lewis, 1996; Wäckers & Lewis, 1999). These methods with little change are still the primary guidelines used today for conducting research on the learning ability of *M. croceipes*. However, recent efforts indicate that some individuals are examining the parameters of these methodologies and their impact on the results generated. For example, the gustatory perception of the unconditioned stimulus employed significantly affects behavioral response of *M. croceipes* to the target volatile (Wäckers et al., 2006).

Less attention has been given to deciphering other, potentially more reliable, observable behaviors that would lead to greater sensitivity in detecting target volatiles with the Wasp Hound. In addition, using an individual behavior to measure the ability of *M. croceipes* to learn and distinguish target odorants from other odorants with similar molecular structure may not be suitable. Consequently, based on other studies that measured a single behavior as in our research, our current understanding of the learning plasticity of *M. croceipes* might be underestimated. Future developments with the Wasp Hound might use multiple behavioral responses, such as antennating and rotation of the body around the exhaust port emitting the air sample. We believe that such an approach potentially could provide a more precise measure of the ability of *M. croceipes* to learn, detect, and respond to target odorants, which could lead to a better understanding of its ecology as well.

The resource type (e.g., food or host), which is different from the quality of the unconditioned stimulus as previously discussed, used to condition an animal to a target volatile can also affect the degree of detection. *Microplitis croceipes* is suspected to have a greater ability to distinguish volatiles associated with hosts rather than food (JK Tomberlin, unpubl.). This sensitivity to molecular differences between target and non-target odorants in association with host might be due to *M. croceipes* having a narrow host range, and the host occurring in a variety of habitats (Fitt, 1989). Basically, a narrow host range increases dependency on select volatiles emitted by host. Without these hosts, propagation of the species will not occur. Therefore, there is a need for *M. croceipes* to be able to learn and identify specific odors associated with that host (Meiners et al., 2002). This could result in more efficient host location under natural circumstances. On the other hand, food resources for *M. croceipes* are more varied and can be more readily located. Therefore, less emphasis is placed on distinguishing an odorant learned in association with food (broad range) vs. when learned in association with hosts (limited range).

However, there is no evidence of a link between ecological factors and learning ability of parasitic wasps so far. Many species of parasitic wasps in different taxonomic groups can learn through association. Examples include the braconids *Cotesia marginiventris* (Cresson) (Tamò et al., 2006), *Cotesia glomerata* (L.), *Cotesia rubecula* (Marshall) (Bleeker et al., 2006), and *Diachasmimorpha longicaudata* (Ashmead) (Segura et al., 2007), as well as the pteromalids *Lariophagus distinguendus* Förster (Müller et al., 2006) and *Nasonia vitripennis* (Walker) (Baeder & King, 2004). Considering that honey bees or social wasps have an acute ability to learn, the ability of parasitic wasps may be an adaptation that cannot be explained by our current level of understanding.

Efforts are underway for the next generation of Wasp Hound to include a more advanced level of behavioral analysis for better qualitative and quantitative assessment of an air sample. Preliminary results with the Wasp Hound indicate that the slope of the area-restricted search response is dependent on the concentration and amount of masking from background odors. Methods to analyze multiple behavioral responses are being investigated to determine if odor concentration can be evaluated from the response of one or more behaviors in the behavioral suite. This development would be extremely useful for aflatoxin detection, which is regulated by the amount found in peanuts, corn, and milk. If odor concentration could be detected as it increases or decreases, the Wasp Hound could also become a tracking sensor, guiding a person or robotic device to an odor source.

References

Alloway TM (1972) Learning and memory in insects. Annual Review of Entomology 17: 42–57.

Baeder JM & King BH (2004) Associative learning of color by males of the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae). Journal of Insect Behavior 17: 201–213.

Bernays EA (1995) Effects of experience on host plant selection. Chemical Ecology of Insects 2 (ed. by RT Cardé & WJ Bell), pp. 44–64. Chapman & Hall, Cincinnati, OH, USA.

Bitterman ME, Menzel R, Fietz A & Schäfer S (1983) Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). Journal of Comparative Psychology 97: 107–119.

Bleckmann H, Schmitz H & von der Emde G (2004) Nature as a model for technical sensors. Journal of Comparative Physiology A 190: 971–981.

Bleeker MAK, Smid HM, Steidle JLM, Kruidhof HM, van Loon JJA & Vet LEM (2006) Differences in memory dynamics between two closely related parasitoid wasp species. Animal Behaviour 71: 1343–1350.

Daly KC & Smith BH (2000) Associative olfactory learning in the moth *Manduca sexta*. Journal of Experimental Biology 203: 2025–2038.

Daly KC, Durtschi ML & Smith BH (2001) Olfactory-based discrimination learning in the moth, *Manduca sexta*. Journal of Insect Physiology 47: 375–384.

Daly KC, Christensen TA, Lei H, Smith BH & Hildebrand JG (2004) Learning modulates the ensemble representations for odors in primary olfactory networks. Proceedings of the National Academy of Sciences of the USA 101: 10476–10481.

Dethier VG (1976) The Hungry Fly, A Physiological Study of the Behavior Associated with Feeding. Harvard University Press, Cambridge, MA, USA.

Drost YC, Lewis WJ & Tumlinson JH (1988) Beneficial arthropod behavior mediated by airborne semiochemicals. V. Influence of rearing method, host plant, and adult experience on host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis*. Journal of Chemical Ecology 14: 1607–1616.

Erber J, Masuhr T & Menzel R (1980) Localization of short-term memory in the brain of the bee, *Apis mellifera*. Physiological Entomology 5: 343–358.

Faber T, Joerges J & Menzel R (1999) Associative learning modifies neural representations of odors in the insect brain. Nature Neuroscience 2: 74–78.

Faber T & Menzel R (2001) Visualizing mushroom body response to a conditioned odor in honeybees. Naturwissenschaften 88: 472–476.

Fitt GP (1989) The ecology of *Heliothis* in relation to agroecosystems. Annual Review of Entomology 34: 17–53.

vonFrisch K (1956) The 'language' and orientation of the bees. Proceedings of the American Philosophical Society 100: 515–519.

Gould JL (1993) Ethological and comparative perspectives on honey bee learning. Insect Learning: Ecological and Evolutionary Perspectives (ed. by DR Papaj & AC Lewis), pp. 18–50. Chapman & Hall, New York, NY, USA.

Habib MK (2007) Controlled biological and biomimetic systems for landmine detection. Biosensors and Bioelectronics 23: 1–18.

Hammer M & Menzel R (1995) Learning and memory in the honeybee. Journal of Neuroscience 15: 1617–1630.

Hammer M & Menzel R (1998) Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. Learning and Memory 5: 146–156.

Herman TJB & Davidson MM (2000) Introduction and establishment of *Microplitis croceipes*, a larval parasitoid of *Heliothis*, in north island pine forests. New Zealand Plant Protection 53: 328–333.

Holland PC (2004) Relations between pavlovian-instrumental transfer and reinforcer devaluation. Journal of Experimental Psychology 30: 104–117.

Kimble GA (1961) Hilgard and Marquis' Conditioning and Learning. Appleton-Centure-Crofts, New York, NY, USA.

King TL, Horine FM, Daly KC & Smith BH (2004) Explosives detection with hard-wired moths. IEEE Transactions on Instrumentation and Measurement 53: 1113–1118.

Le HK & Takasu K (2005) *Helicoverpa armigera* as an alternative host of the larval parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). Applied Entomology and Zoology 40: 679–686.

Lewis WJ (1970) Life history and anatomy of *Microplitis croceipes* (Hymenoptera: Braconidae), a parasite of *Heliothis* spp. (Lepidoptera: Noctuidae). Annals of the Entomological Society of America 63: 67–70.

Lewis WJ & Martin WR Jr (1990) Semiochemicals for use with parasitoids: status and future. Journal of Chemical Ecology 16: 3067–3089.

Lewis WJ & Tumlinson JH (1988) Host detection by chemically mediated associative learning in a parasitic wasp. Nature 331: 257–259.

Lieberman DA (1993) Learning: Behavior and Cognition. Brooks/Cole Publishing Company, Pacific Grove, CA, USA.

Meiners T, Wäckers F & Lewis WJ (2002) The effect of molecular structure on olfactory discrimination by the parasitoid *Microplitis croceipes*. Chemical Senses 27: 811–816.

Meller VH & Davis RL (1996) Biochemistry of insect learning: Lessons from bees and flies. Insect Biochemistry and Molecular Biology 26: 327–335.

Müller C, Collatz J, Wieland R & Steidle JLM (2006) Associative learning and memory duration in the parasitic wasp *Lariophagus distinguendus*. Animal Biology 56: 221–232.

Olson DM, Rains GC, Meiners T, Takasu K, Tumlinson JH & Lewis WJ (2003) Parasitic wasps learn and report diverse chemicals with unique conditionable behaviors. Chemical Senses 28: 545–549.

Papaj DR & Prokopy RJ (1989) Ecological and evolutionary aspects of learning in phytophagous insects. Annual Review of Entomology 34: 315–350.

Park KC, Ochieng SA, Zhu J & Baker TC (2002) Odor discrimination using insect electroantennogram responses from an insect antennal array. Chemical Senses 27: 343–352.

Pavlov I (1927) The Conditioning Reflex. WW Norton, New York, NY, USA.

Prokopy RJ & Lewis WJ (1993) Application of learning to pest management. Insect Learning: Ecological and Evolutionary Perspectives (ed. by DR Papaj & AC Lewis), pp. 308–342. Chapman & Hall, New York, NY, USA.

Quinn WG, Harris WA & Benzer S (1974) Conditioned behavior in *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of the USA 71: 708–712.

Rains GC, Tomberlin JT, D'Alessandro M & Lewis WJ (2004) Limits of volatile chemical detection of a parasitoid wasp, *Microplitis croceipes*, and an electronic nose: a comparative study. Transactions of the ASAE 47: 2145–2152.

Rains GC, Utley SL & Lewis WJ (2006) Behavioral monitoring of trained insects for chemical detection. Biotechnology Progress 22: 2–8.

Sandoz JC, Galizia CG & Menzel R (2003) Side-specific olfactory conditioning leads to more specific odor representation between sides but not within sides in the honeybee antennal lobes. Neuroscience 120: 1137–1148.

Segura DF, Viscarret MM, Carabajal Paladino LZ, Ovruski SM & Cladera JL (2007) Role of visual information and learning in habitat selection by a generalist parasitoid foraging for concealed hosts. Animal Behaviour 74: 131–142.

Skinner BF (1953) Science and Human Behavior. McMillan, New York, NY, USA.

Stephens DW (1993) Learning and behavioral ecology: incomplete information and environmental predictability. Insect Learning: Ecological and Evolutionary Perspectives (ed. by DR Papaj & AC Lewis), pp. 195–218. Chapman & Hall, New York, NY, USA.

Suh GSB, Wong AM, Hergarden AC, Wang JW, Simon AF et al. (2004) A single population of olfactory sensory neurons mediates an innate avoidance behaviour in *Drosophila*. Nature 431: 854–859.

Takasu K & Lewis WJ (1993) Host- and food-foraging of the parasitoid *Microplitis croceipes*: learning and physiological state effects. Biological Control 3: 70–74.

Takasu K & Lewis WJ (1995) Importance of adult food sources to host searching of the larval parasitoid *Microplitis croceipes*. Biological Control 5: 25–30.

Takasu K & Lewis WJ (1996) The role of learning in adult food location by the larval parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae). Journal of Insect Behavior 9: 265–281.

Takasu K & Lewis WJ (2003) Learning of host searching cues by the larval parasitoid *Microplitis croceipes*. Entomologia Experimentalis et Applicata 108: 77–86.

Takasu K, Rains GC & Lewis WJ (2007) Comparison of detection ability of learned odors between males and females in the larval parasitoid *Microplitis croceipes*. Entomologia Experimentalis et Applicata 122: 247–251.

Tamò C, Ricard I, Held M, Davison AC & Turlings TCJ (2006) A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours. Animal Biology 56: 205–220.

Thorpe WH (1963) Learning and Instinct in Animals, 2nd edn. Harvard University Press, Cambridge, MA, USA.

Tomberlin JK, Tertuliano M, Rains GC & Lewis WJ (2005) Conditioned *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) detect and respond to 2,4-DNT: development of a biological sensor. Journal of Forensic Sciences 50: 1187–1190.

Tully T (1984) *Drosophila* learning: behavior and biochemistry. Behavior Genetics 14: 527–557.

Tully T & Quinn WG (1985) Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. Journal of Comparative Physiology A 157: 263–277.

Turlings TCJ, Wäckers FL, Vet LEM, Lewis WJ & Tumlinson JH (1993) Learning and host-finding cues by hymenopterous parasitoids. Insect Learning: Ecological and Evolutionary Perspectives (ed. by DR Papaj & AC Lewis), pp. 51–78. Chapman & Hall, New York, NY, USA.

Vass AA, Barshick S-A, Sega G, Caton J, Skeen JT & Love JC (2002) Decomposition chemistry of human remains: a new methodology for determining the postmortem interval. Journal of Forensic Sciences 47: 542–553.

Vass AA, Smith RR, Thompson CV, Burnett MN, Wolf DA et al. (2004) Decompositional odor analysis database. Journal of Forensic Sciences 49: 760–769.

Vet LEM, Lewis WJ & Cardé RT (1995) Parasitoid foraging and learning. Chemical Ecology of Insects 2 (ed. by RT Cardé & WJ Bell), pp. 65–101. Chapman & Hall, New York, NY, USA.

Wäckers FL & Lewis WJ (1999) A comparison of color-, shape- and pattern-learning by the hymenopteran parasitoid *Microplitis croceipes*. Journal of Comparative Physiology 184: 387–393.

Wäckers FL, Bonifay C & Lewis WJ (2002) Conditioning of appetitive behavior in the hymenopteran parasitoid *Microplitis croceipes*. Entomologia Experimentalis et Applicata 103: 135–138.

Wäckers FL, Bonifay C, Vet L & Lewis WJ (2006) Gustatory response and appetitive learning in *Microplitis croceipes* in relation to sugar type and concentration. Animal Biology 56: 193–203.

Wang S, Zhang S, Sato K & Srinivasan MV (2005) Maturation of odor representation in the honeybee antennal lobe. Journal of Insect Physiology 51: 1244–1255.

Zars T, Fischer M, Schulz R & Heisenberg M (2000) Localization of a short-term memory in *Drosophila*. Science 288: 672–675.