Associative Learning in Insects:
Evolutionary Models, Mushroom Bodies, and a Neuroscientific Conundrum

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Environmental predictability has for many years been posited to be a key variable in whether learning is expected to evolve in particular species, a claim revisited in two recent papers. However, amongst many researchers, especially neuroscientists, consensus is building for a very different view, namely that learning ability may be an emergent property of nervous systems and, thus, all animals with nervous systems should be able to learn. Here we explore these differing views, sample research on associative learning in insects, and review our own work demonstrating learning in larval antlions (Neuroptera: Myrmeleontidae), a highly unlikely insect candidate. We conclude by asserting that the capacity for associative learning is the default condition favored by neuroscientists: Whenever selection pressures favor evolution of nervous systems, the capacity for associative learning follows *ipso facto*. Nonetheless, to reconcile these disparate views, we suggest that (a) models for the evolution of learning may instead be models for conditions overriding behavioral plasticity; and, (b) costs of learning in insects may be, in fact, costs associated with more complex cognitive skills, skills that are just beginning to be discovered, rather than simple associative learning.

Keywords: insect learning, invertebrate learning, associative learning, insect behavior, antlions, Neuroptera, Myrmeleontidae, sit-and-wait predation

Two recent papers highlight not only the attention that associative learning in insects continues increasingly to receive, but also the conundrum that lies at the heart of attempts to account for the evolution of learning, a topic that has generated very different viewpoints amongst behavioral ecologists and neuroscientists. In one paper (Farris & Schulmeister, 2011), the authors very effectively and carefully dismantle what has been called the "social brain hypothesis"—at least insofar as it pertains to hymenopteran insects, such as bees, ants and wasps—namely the notion that the ability to engage in complex social behavior drove the evolutionary expansion of brain size. Instead, Farris and Schulmeister argue, the cognitive demands imposed by parasitoid, host-finding behavior drove the observed brain expansion. That is, because parasitoidism requires searching for hosts (often other insects) that are, themselves, mobile in a constantly changing environment, the need for associative and spatial learning mechanisms to help guide that difficult search was responsible for greatly enlarged and morphologically elaborate brain structures called mushroom bodies. Although all insects possess mushroom bodies, a pair of aptly named hemispherical structures within the brain that sit atop an elongated stalk, or *peduncle*, mushroom bodies greatly differ in size and architecture between different species (Strausfeld, Hansen, Li, Gomez & Ito, 1998). According to Farris & Schulmeister, more elaborate mushroom bodies would have enabled the ancestors of present-day Hymenoptera, insects that are, notably, the focus of so much associative learning research, to expand beyond their dependence on plants as hosts for

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their developing eggs and instead adopt a parasitoid life history in which the bodies of other living organisms serve as egg hosts. Evidence for their hypothesis comes from a very detailed phylogenetic analysis involving a quantitative and qualitative survey of brain structure in extant hymenopteran species, which reveals that parasitoidism evolved concurrently with the elaborate mushroom body architecture, and predated the advent of sociality by approximately 90 million years.

Although the focus of the Farris & Schulmeister (2011) article is less on the evolution of learning, per se, than on the notion that parasitoidism, rather than sociality, drove the expansion of brain architecture, the authors clearly are making the case that the demands of searching in a changing environment are key to the evolution of associative learning mechanisms. As they put it, "for insects that must navigate among multiple distant sites"—and here they acknowledge that the search could involve not just hosts but also food and mates—"…the processing demands of spatial learning may have promoted the evolution of larger mushroom bodies with novel circuits for processing visual information and forming associative and spatial memories (p.8)."

Amongst many behavioral ecologists, too, environmental predictability (or its converse, environmental stability) has, for a long time, been posited to be a key variable in whether learning is expected to evolve in a particular species (Dukas, 1998; Johnston, 1982; Kerr & Feldman, 2003; Papaj, 1994; Stephens, 1991, 1993). Recently, Dunlap and Stephens (2009) have developed an elegant, and easily testable, mathematical model for the evolution of learning. In their model, they carefully tease apart two variables that combine to influence the fitness value of learning. One variable is the reliability of an animal's experience with its environment, that is, the extent to which the environment is predictable, and environmental cues are reliable. A second variable is the reliability of a fixed behavior pattern, that is, the extent to which a given behavior produces a reliably favorable outcome. Using a preparation developed by Mery and Kawecki (2002, 2004) in which successive generations of *Drosophila* were tested for their ability to learn, Dunlap and Stephens demonstrate that the learning phenotype emerges only when the predictability of the natural environment is greater than the reliability of a fixed behavior pattern. Put another way, if a fixed behavior strategy results in a mostly favorable outcome, then evolution should favor that behavior pattern rather than the more plastic behavior generated by learning. If, however, the environment is both variable *and* predictable, then environmental variability makes fixed behavior less advantageous, at the same time that its predictability permits a reliable learning strategy.

Taken at face value, each of these two papers would seem to suggest that a review of the associative learning literature in insects would yield, at the very minimum, distinctive patterns of learning abilities across insect species, with some species showing little to no associative learning capacity. For example, if we extend Farris and Schulmeister's (2011) argument to what appears to be its logical conclusion, the ability to form "*associative* [emphasis added] and spatial memories" requires complex circuitry, circuitry that some insects, namely those without elaborated mushroom bodies, are not likely to possess. Extrapolating from Farris and Schulmeister's data, in which they demonstrate that only parasitoid hymenopterans, and not phytophagous (i.e., plant eating) species, possess highly elaborated mushroom bodies, independent of whether they are social, eusocial or solitary, we should expect to find the same phylogenetic pattern of learning and memory abilities across hymenopteran insects. That is, at least some phytophagous hymenoptera—and, one would assume, at least some phytophagous insects of other insect orders—would be expected to exhibit diminished learning capacities, at best. As we show below, however, the associative learning data would not seem to support this conclusion. Likewise (indeed, perhaps even more so), Dunlap & Stephens' (2009) model, which describes a combination of conditions that favor a non-learning phenotype, predicts that associative learning abilities would not be present in all insects, either because the conditions for learning never existed, or because the capacity was secondarily lost whenever non-learning conditions arose (as they demonstrated in *Drosophila melanogaster*). At the very minimum, then, some insect species should possess either a greatly diminished capacity for associative learning, or no capacity to learn at all. Again, however, as our brief sample of the recent literature in the next section makes clear, associative learning appears to be universal within insects.

In the remainder of this paper, we provide a very brief snapshot of learning in insects, followed by a more detailed review of our own research demonstrating learning in larval antlions, an insect that once would have been considered a highly unlikely candidate for associative learning. In our conclusion, we argue that the capacity for associative learning is almost certainly an emergent property of all neural circuitry, the view espoused by neuroscientists. Nonetheless, we attempt to reconcile what seem to be disparate views of insects' capacity to learn by suggesting two critical modifications to the arguments made by Dunlap and Stephens (2009) and Farris and Schulmeister (2011): One, we propose that models for the evolution of learning, such as the one proposed by Dunlap and Stephens, but not limited to theirs alone, may in fact be models for the conditions under which plasticity is overridden. That is, under certain conditions, selection pressures may override the universal ability of insects to learn, resulting in hard-wired, or considerably less

plastic, responses. Two, insofar as the function of elaborate mushroom bodies are concerned (e.g., Farris & Schulmeister, 2011), we propose that any differences between insects in their cognitive capacities is not likely to be at the level of associative learning, but rather involve far more complex cognitive feats. In short, we've been vastly underestimating insects' cognitive abilities: As some very recent work has shown (e.g., Avarguès-Weber, Dyer, & Giurfa, 2011; Chittka & Geiger, 1995; Dacke & Srinivasan, 2008; Giurfa, Zhang, Jenett, Menzel & Srinivasan, 2001) elaborate mushroom bodies can do far more than we've been giving them credit.

Learning in Insects

In this section we describe some of the more recent studies of insect learning, a sample of the literature that is intended to illustrate the diversity of insects studied, as well as the diversity of ways in which associative learning is employed by insects, rather than an exhaustive review of this voluminous literature: Not only is a review of such breadth and depth far beyond the scope of this paper, but also many excellent reviews already exist (Dukas, 2008; North & Greenspan, 2007; Papaj, 2003; Papaj & Lewis, 1993) Nonetheless, as we hope to demonstrate, long gone is the notion that insects are little more than automatons, reacting to environmental stimuli with innate, fixed behavioral patterns (Wheeler, 1930). Beginning over 40 years ago with some of the very first studies of honeybees, *Apis mellifera* (e.g., Menzel, 1968; Menzel, Erber & Masuhr, 1974), fruit flies, *Drosophila melanogaster* (e.g., Murphy, 1967, 1969; Quinn, Harris & Benzer, 1974; Spatz, Emanns & Reichert, 1974), and ants, *Formica* spp. (Schneirla, 1941, 1943), learning continues to be documented in a wide variety of social, eusocial, and solitary-living insects, spanning multiple families in all major insect orders (Guillette & Hollis, 2010; North & Greenspan, 2007; Papaj, 2003; see Figure 1). Indeed, recent experiments suggest that insects are capable of even much more than forming simple associations between a cue and a response: They can learn to discriminate between multiple cues, retain the information over long periods of time, and transfer learning to completely new environments.

For example, cockroaches, *Periplaneta americana* (Order Blattodea), are capable of discriminating between two odors, one associated with a food reward and another associated with an aversive taste stimulus (Wantanabe, Kobayashi, Sakura, Matsumoto & Mizunami, 2003). Moreover, they are able to transfer this learning from a very artificial situation in which they were immobilized for purposes of training, to another, completely different, semi-naturalistic environment. Finally, they were also capable of retaining the discrimination over a four-day test phase.

In a demonstration of an even longer, and somewhat more dramatic retention test, first instar moth larvae, *Cydia pomonella* (Order Lepidoptera), were fed a small amount of a noxious plant, which either had been flavored with saccharine or had not been flavored (Pszczolkowski & Brown, 2005). In a subsequent two-choice test, larvae that previously were exposed to the flavored plant avoided burrowing in apples that had been flavored with saccharine, even though apples are their preferred host fruit. Of greater importance, however, this learned food aversion was exhibited by trained moths even after they had molted and emerged as second instar larvae.

Another study examined the retention of olfactory learning across pupation in the aphid parasitoid wasp, *Aphidius ervi* Haliday (Order Hymenoptera) (Gutiérrez-Ibáñez, Villagra & Niemeyer, 2007). Third instar larvae typically bite through the exoskeleton of their dead aphid hosts and thus are exposed to olfactory cues from the local environment. In a preliminary experiment, third instar larvae were placed on a broad bean plant leaf prior to cocooning, and therefore were exposed to the olfactory cues of the plant. Larvae in a second group were placed on a plastic plate prior to cocooning, and therefore were exposed to no odor. After the adult wasps emerged, they were tested for odor preference in a two-choice Y-maze. Adults that cocooned on the plant showed a significant preference for the plant odor, while subjects that cocooned on the plastic showed no preference. Because, one might argue, environmental cues from the larval environment could have carried over to the adult environment as the adult emerged from the cocoon, and thus learning of the olfactory cue could have taken place during the adult stage rather than during the larval stage, a second experiment explored this alternative explanation in a less naturalistic, but better controlled design: One group of third instar larvae was exposed to vanilla odor after biting through the aphid carcass, whereas the control group was exposed to water vapor. Subsequently, half of the larvae in each group were removed from their cocoon, to avoid carryover of odor from the larval training phase. Adults that were exposed to the vanilla odor as larvae exhibited a significantly greater preference for vanilla odor compared to larvae in the control group, regardless of whether or not they were removed from their cocoons before emerging as adults; however, adults in the control group, which were exposed to water vapor as larvae, showed no such preference. Thus, this study clearly demonstrates that learned preferences for host plant odors are not due to a "chemical legacy" (*sensu* Corbet, 1985) carryover, but are due, instead, to learning that occurred in the larval stage, which was retained across pupation.

Transfer of learning also has been demonstrated in another parasitoid wasp, the tiny *Microplitis croceipes* (Order Hymenoptera), which is capable of using learned odor cues to locate food at the astonishing sensitivity of four parts per bil-

lion (Rains, Utley & Lewis, 2006). In a transfer experiment (Lewis & Takasu, 1990), wasps first were made hungry and then were trained to associate one odor with food and another, different, odor with a host source. Next, they were tested for their odor preference after being well fed. Demonstrating their ability to transfer learning across changing bodily states and to respond in terms of their current need, wasps flew to the odor associated with the host source (Lewis & Takasu, 1990).

Different bee species, all hymenopterans, long have been subjects in associative learning experiments, of course, and, among them, honeybees have served as a model for studying the panoply of traditional associative learning phenomena, including extinction and spontaneous recovery, compound and context conditioning, as well as blocking, overshadowing, and various inhibitory phenomena (e.g., Bitterman, Menzel, Fietz & Schäfer, 1983; Blaser, Couvillon & Bitterman, 2006; Couvillon & Bitterman, 1980; Couvillon, Hsiung, Cooke & Bitterman, 2005). A slightly different approach has expanded our understanding of several of these associative learning phenomena by examining behavior at both behavioral and neuronal levels (e.g., Arenas, Giurfa, Farina & Sandoz, 2009; Lachnit, Giurfa & Menzel, 2004). For example, Arenas, Giurfa, Farina and Sandoz (2004) exposed honeybees, *Apis mellifera*, on Days 5-8 post-emergence from the egg, to a particular odor while they were feeding; bees in the control group received no odor training. Bees then were tested on Day 17 for a proboscis extension response (PER) to the training odor, and three additional test odors. In the treatment group, a significantly higher PER was elicited by the training odor and a perceptually similar test odor, but not the two other test odors. The four test odors did not elicit differential amounts of PER in the control group. A similar pattern of results emerged when the activity patterns of glomeruli (synaptic waystations) in the antennal lobe, elicited by the four different odors, were examined: That is, in the treatment group, the two most perceptually similar odors elicited the most highly correlated activity; no such correlations in neuronal activity were detected in control bees. Finally, another recent study of hymenopteran bees demonstrates (rather famously, as the study was performed by 25 8-10-year-old children and published in *Biology Letters*) that bumblebees, *Bombus terrestris*, can use complex color *patterns* (i.e., not just color alone) to guide their search for food (Blackawton et al., 2011).

In many associative learning studies of insects, either the training environment, or the testing environment, or both, are far removed from the animal's natural environment. Of course, the point of much of this work is not to explore the adaptive value of learning, per se, but to demonstrate learning in a novel insect species. Nonetheless, numerous demonstrations of adaptive value can be found. For example,

in one experiment, grasshoppers, *Schistocerca americana* (Order Orthoptera), searched for food in an environment that contained food differing in nutritional quality (Dukas & Bernays, 2000). For one group of grasshoppers, those in the associative learning group, the spatial location, taste and color associated with high quality food remained consistent throughout training. For grasshoppers in the control group, these cues varied randomly in relation to the high quality food. Over the course of this experiment, grasshoppers in the associative learning condition not only visited the high quality food site more often than grasshoppers in the random control group, but also they spent more time eating the high quality food. More importantly, grasshoppers that were able to use learned cues to guide their food search grew at a faster rate than controls, not only exhibiting 15% higher fat mass and 11% higher non-fat mass, but pupating sooner; that is, the duration of their final instar was 7% shorter. Because previous studies in grasshoppers (Atkinson & Begon, 1987) have shown that larger body mass is positively correlated with the number and size of eggs laid, and that less time spent travelling to obtain quality food translates into a lower predation risk, the adaptive value of using learned cues to guide food search is clear.

A series of similar studies in locusts, *Locusta migratoria*, another orthopteran representative in the grasshopper family, were designed to explore how these plant-eating insects could use associatively learned cues to guide their food choice. When, for example, locusts were made selectively deficient in either carbohydrate or protein intake, they were able to use color cues (Raubenheimer & Tucker, 1997)—and, albeit to a somewhat more limited extent, odor cues (Simpson & White, 1990)—to guide them to a food source previously paired with a diet that contained an adequate amount of that specific nutrient. Locusts also are capable of learned taste aversions; that is, they are able to avoid novel plant foods that are followed by injections of either lithium chloride, the classic poison used in vertebrate aversion studies, or common toxic plant compounds (Lee & Bernays, 1990). Finally, these same animals can use visual cues to guide their search for water, an equally important aspect of the ingestive behavior of herbivorous insects (Raubenheimer & Blackshaw, 1994).

The adaptive value of learning also was explored in a series of experiments with parasitoid wasps (*Biosteres arisanus*, Order Hymenoptera; Dukas & Duan 2000). Subjects in the learning group were given the opportunity to form an association between the types of fruit that contained host eggs, while subjects in the control group were not given this advantage. During a two-choice test, wasps in the learning group correctly choose the type of fruit that contained host eggs at a high rate (~80-90%) over a two-day period, demonstrating long-term retention of learned associations. The

Figure 1. Associative Learning in Insects (Class: Insecta)

Order Name (Common Name)	Number of Families/Species		Families Studied	Selected Examples of Associative Learning
Archaeognatha (bristletails)	2	500	0	None studied
Zygentoma (silverfish)	5	400	Lepismatidae	· *Lepisma saccharina* (silverfish) – Punzo (1980)
Ephemeroptera (mayflies)	40	3,100	0	None studied
Odonata (dragonflies, damselflies)	33	5,600	Coenagrionidae	· *Enallagma* spp. (damselfly) – Wisenden, Chivers & Smith (1997)
Blattodea (cockroaches)	5	4,000	Blaberidae Blattidae	· *Leucophaea maderae* (Madeira cockroach) – Decker, McConnaughey & Page (2007) · *Periplaneta americana* (American cockroach) – Wantanabe, Kobayashi, Sakura, Matsumoto & Mizunami (2003)
Mantodea (mantids)	8	1,800	Mantidae	· *Tenodera ardifolia* (mantid) – Bowdish & Bultman (1993)
Isoptera (termites, white ants)	7	2,500	0	None studied
Grylloblattodea (rock crawlers)	1	75	0	None studied
Dermaptera (earwigs)	7	2,000	0	None studied
Plecoptera (stoneflies)	16	2,000	Perlidae	· *Paragnetina media* (stonefly) – Feltmate & Williams (1991)
Embiidina (webspinners)	8	300	0	None studied
Orthoptera (grasshoppers, katydids)	29	24,000	Acrididae Gryllidae	· *Locusta migratoria* (migratory locust) – Raybenheimer & Tucker (1997); Simpson & White (1990) · *Melanoplus sanguinipes* (grasshopper) – Bernays & Wrubel (1985) · *Shistocerca americana* (American desert locust) – Dukas & Bernays (2000) · *Shistocerca gregaria* (desert locust) – Behmer, Belt & Shapiro (2005) · *Gryllus bimaculatus* (field cricket) – Matsumoto & Mizunami (2004); Lyonsa & Barnard (2006)
Phasmida (walkingsticks)	2	3,000	0	None studied
Mantophasmatodea (gladiators, heel-walkers)	1	16	0	None studied
Zoraptera (angel insects)	1	32	0	None studied
Psocoptera (booklice, barklice)	17	4,400	0	None studied
Phthiraptera (biting lice, sucking lice)	24	4,900	0	None studied
Hemiptera (true bugs)	104	55,000	Cicadellidae	· *Homalodisca vitripennis* (glassy-winged sharpshooter) – Patt & Setamou (2010)
Thysanoptera (thrips)	9	6,000	0	None studied
Megaloptera (alderflies, dobsonflies)	2	328	0	None studied
Raphidioptera (snakeflies)	2	215	0	None studied
Neuroptera (lacewings, antlions)	17	6,000	Myrmeleontidae	· *Myrmeleon crudelis* (antlion) – Guillette, Hollis & Markarian (2009); Hollis, Cogswell, Snyder, Guillette & Nowbahari (2011)
Coleoptera (beetles)	135	350,000	Elateridae Tenebrionidae	· *Limonius canus* (Pacific Coast wireworm) – Van Herk, Vernon, Harding, Roitberg & Gries (2010; but see reference for cautionary note) · *Tenebrio molitor* (mealworm beetle) – Alloway (1972) · *Tenebrio obscurus* (darkling beetle) – Punzo & Malatesta (1988)
Strepsiptera (twisted-wing parasites)	8	550	0	None studied
Mecoptera (scorpion flies)	9	570	0	None studied

Figure 1. Associative Learning in Insects (Class: Insecta) continued

Order Name (Common Name)	Number of Families/Species		Families Studied	Selected Examples of Associative Learning
Diptera (flies)	117	150,000	Calliphoridae	· *Lucilia cuprina* (walking blowfly) – Campbell & Strausfeld (2001) · *Phormia regina* (black blowfly) – McGuire (1984) · *Protophormia terraenovae* (blue-bottlefly) – Sokolowski, Disma, Abramson (2010)
			Culicidae	· *Anopheles gambidae* (malaria vector mosquito) – Seger (2010; but see text for cautionary note) · *Aedes aegypti* (yellow fever vector mosquito) – Kaur, Lai & Giger (2003; but see text for cautionary note) · *Culex quinquefasciatus* (filariasis vector mosquito) – Tomberlin, Rains, Allan, Sanford & Lewis (2006)
			Drosophilidae	· *Drosophila melanogaster* (fruitfly) – See Busto, Cervantes-Sandoval & Davis (2010) for a review
			Muscidae	· *Musca domestica* (house fly) – McGuire (1984)
			Tachinidae	· *Exorista mella* (tachinid fly) – Stireman (2002) · *Drino bohemica* (tachinid fly) – Monteith (1963)
			Tephritidae	· *Rhagoletis pomonella* (apple maggot fly) – Prokopy, Reynolds & Ent (1998; but see reference for cautionary note)
Siphonaptera (fleas)	15	2,600	Pulicidae	· *Xenopsylla conformis* (rat flea) – Hawlena, Abramsky & Krasnov (2007)
Lepidoptera (moths, butterflies)	120	160,000	Arctiidae	· *Diacrisia virginica* (wooly bear caterpillar) – Dethier (1980) · *Estigmene congrua* (wooly bear caterpillar) – Dethier (1980)
			Nymphalidae	· *Danaus plexippus* (monarch butterfly) – Rodrigues, Goodner & Weiss (2010)
			Papilionidae	· *Battus philenor* (pipevine swallowtail) – Weiss (1997); Allard & Papaj (1997)
			Pieridae	· *Pieris rapae* (small cabbage white butterfly) – Snell-Rood & Papaj (2009) · *Pieris brassicae* (large cabbage white butterfly) – Smallegange, Everaarts & van Loon (2006)
			Sphingidae	· *Manduca sexta* (tobacco hornworm) – Blackiston, Silva, Casey & Weiss (2008)
			Tortricidae	· *Cydia pomonella* (codling moth) – Pszczolkowski & Brown (2005)
Trichoptera (caddisflies)	46	13,000	0	None studied
Hymenoptera (ants, bees, wasps)	73	150,000	Apidae	· *Apis mellifera* (European honey bee) – Menzel (1968); Blazer, Couvillon & Bitterman (2006); Arenas, Giurfa, Farina & Sandoz (2009) · *Bombus terrestris* (bumblebee) – Blackawton et al. (2010)
			Braconidae	· *Aphidius ervi* (parasitoid wasp) – Gutiérrez-Ibáñez, Villagra & Niemeyer (2007) · *Microplitis croceipes* (parasitoid wasp) – Rains, Utley & Lewis (2006); Lewis & Takasu (1990) · *Biosteres arisanus* (parasitoid wasp) – Dukas & Duan (2000) · *Asobara* ssp. (parasitoid wasp) – Vet & van Opzeeland (1984) · See Stireman (2002) for many additional species
			Eucoilidae	· *Leptopilina heterotoma* (parasitoid wasp) – Papaj, Snellen, Swaans & Vet, (1994)
			Figitidae	· *Leptopilina boulardi* (parasitoid wasp) – Kaiser, Pérez-Maluf, Sandoz, & Pham-Delègue (2003)
			Formicidae	· *Formica* spp. (ant) – Schneirla (1941) · *Camponotus aethiops* (carpenter ant) – Guerrieri & d'Ettorre (2010)
			Ichneumonidae	· *Itoplectis conquisitor* (parasitic wasp) – Arthur (1966) · *Exeristes roborator* (parasitic wasp) – Wardle (1990) · *Venturia canescens* (parasitic wasp) – Desouhant, Navel, Foubert, Fischbein, Thety & Bernstein (2010)
			Pteromalidae	· *Nasonia vitripennis* (parasitoid wasp) – Oliai & King (2000); Baeder & King (2004)

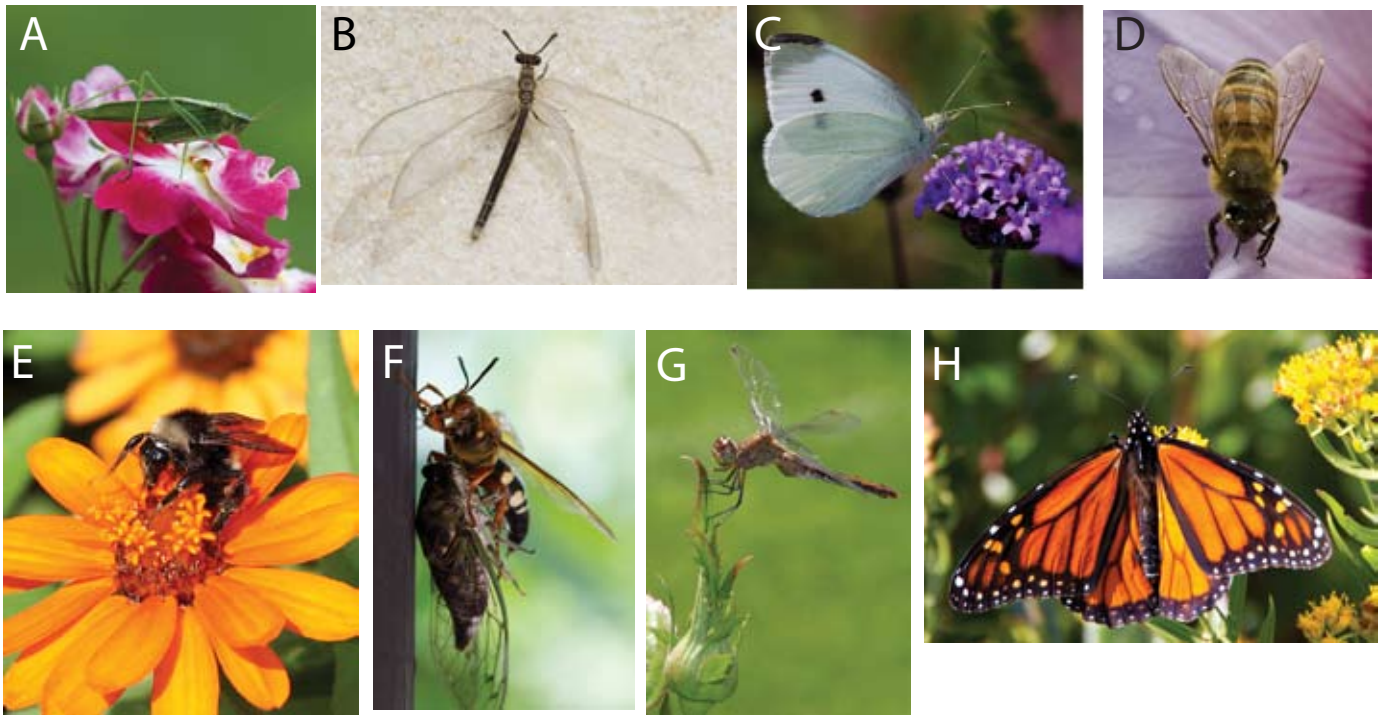## Figure 1. Associative Learning in Insects continued

Figure 1. *Learning in insects, a single class of organisms within the superclass Hexapoda (from the Greek, literally "six feet"). The insect orders listed here, as well as the estimated number of families and species in that order, are those described by Resh and Cardé (2009). In this figure, we have attempted to name all families in which the associative learning capacity of at least one species has been studied; rows in which no family name is listed are those in which, to our best knowledge, no one has claimed to demonstrate associative learning (with appropriate associative controls) in any member of that insect order. For example, associative learning has not been demonstrated in bristletails, Archaeognatha, the first order listed above. This figure is not intended to be an exhaustive list of all papers reporting associative learning in insects. We provide no more than two references for any single insect species, and no more than a few species within a single family. Nonetheless, the figure is designed to expand the information provided in the text, illustrating both the diversity of associative learning in the Class Insecta, as well as obvious gaps in our knowledge. Photography courtesy of Cheryl McGraw: A. katydid (Orthoptera: Tettigoniidae); B. adult antlion (Neuroptera: Myrmeleontidae); C. cabbage white butterfly, Pieris rapae (Lepidoptera: Pieridae); D. honeybee, Apis spp. (Hymenoptera: Apidae); E. bumblebee, Bombus spp. (Hymenoptera: Apidae); F. a female eastern cicada killer wasp, Sphecius speciosus (Hymenoptera: Crabronidae) in the process of using her stinger to paralyze a cicada (Hemiptera: Cicadidae); G. dragonfly, (Odonata: Anisoptera); and, H. monarch butterfly, Danaus plexippus (Lepidoptera: Nymphalidae).*

correct choice of the fruit which contained hosts translated into a significant fitness advantage for wasps in the learning group. Compared to control wasps, not only were learning wasps able to parasitize more host eggs, but more of their offspring reached adulthood.

Finally, a possible newcomer to the list of insects capable of associative learning may be the malaria vector mosquito, *Anopheles gambiae* (Order Diptera); however, much additional work is needed before firm conclusions may be drawn (Seger, 2010). Although several earlier studies claimed to have demonstrated learning in various mosquito species (e.g., Kaur, Lai & Giger, 2003), a careful review of this work (Alonso & Schuck-Paim, 2006) reveals that various methodological problems render those data inconclusive.

The analysis conducted by Alonso and Schuck-Paim (2006) has broader implications, implications that extend far beyond whether mosquitoes should be added to the list. In short, not all studies, with mosquitoes as well as with other insects, include the requisite associative learning control group. This important group experiences both the cue and the biologically important event that the cue is intended to signal in the experimental group, but which are not paired explicitly as they are in the experimental group. Without this group, habituation, sensitization or pseudoconditioning may

be responsible for the learned behavior. To be sure, some form of learning, in the broadest sense of that term, is not disputed, only whether associative learning, per se, is responsible. Nonetheless, as our own search of the literature has made clear to us, no sooner does one study become disputed than another study, with the same or a closely related insect species, appears. To give but one example, Tomberlin, Rains, Allan, Sanford and Lewis (2006) have demonstrated that another mosquito, *Culex quinquefasciatus*, is capable of true associative learning, namely the ability to associate an odor with either a sugar- or blood-meal.

Whether or not additional mosquito species are added to the list, however, the work that we sample here, together with countless other studies, too numerous to mention, clearly demonstrate that associative learning in insects is common. Certainly, as revealed in Figure 1, many of the species chosen for study are parasitoid hymenoptera, as predicted by Farris and Schulmeister's (2010) theory of how mushroom bodies evolved to meet the cognitive demands of host-finding behavior guided by associative and spatial learning. However, not all subject species are parasitoids, as our brief review reveals. Indeed, some of the more complex learning abilities in insects has been demonstrated in phytophagous (plant-eating) members of the grasshopper family, as described above (e.g., Dukas & Bernays, 2000; Papaj & Prokopy, 1989; Raubenheimer & Tucker, 1997; Simpson & White, 1990). Nonetheless, one might argue that, parasitic or not, each species that demonstrates a capacity for learning is also one that is forced to search in an unpredictable environment and, thus, the "environmental unpredictability" models for the evolution of learning still might hold true. That is, prior to our own work with larval antlions (Guillette & Hollis, 2010; Guillette, Hollis & Markarian, 2009; Hollis, Cogswell, Snyder, Guillette & Nowbahari, 2011), all studies of insect learning had one fundamental feature in common: Each of the many insect species that had been chosen for study is one that moves about its environment as it actively seeks food, locates a host, evades a parasite, or avoids some noxious stimulus in its environment. Associative learning, then, provides a powerful mechanism for guiding what would otherwise be a difficult search. However, our experiments with larval antlions, which belong to the order Neuroptera, a group of "net-winged" insects that includes lacewings, suggest that neither searching nor environmental unpredictability is critical to the ability to learn.

Associative Learning in Larval Antlions

Trap Constructing Predators and the Special Case of an Extreme Sit-and-Wait Strategy

Trap constructing sit-and-wait predators provide an ex-

cellent opportunity to test the notion either that the need to search in an unpredictable environment, or that unpredictability per se, is critical to the ability to learn. Sit-and-wait predators, unlike active foragers, do not seek out and hunt prey. Rather, to capture their prey, sit-and-wait predators either lie in ambush or construct traps (Alcock, 1972), and then rely on the prey to move within the range of their sensory receptors, which in turn communicate with higher level mechanisms to initiate a capture attempt (Bailey, 1998). Two important adaptations help sit-and-wait predators deal with highly variable prey encounters: One, they typically possess elaborate sensory structures to detect their prey well in advance of an actual capture attempt (e.g., Coddington & Levi, 1991; Mencinger-Vračko & Devetak, 2008); and, two, they have evolved very low resting metabolisms and thus can survive extremely long periods without consuming prey (Mansell, 1999; Porges, Riniolo, McBride & Campbell, 2003).

The construction of traps as a method of sit-and-wait predation is not taxonomically widespread, with the majority of trap-builders being spiders (Ruxton & Hansell, 2009). However, in contrast to constructing traps with self-produced and secreted materials (e.g., the silk used to construct spider webs), some species of larval antlions (Neuroptera: Myrmeleontidae; see Figure 2) dig conical pit traps in dry, sandy substrate (Mansell, 1999). Pit-digging antlions, the larvae of neuropteran (i.e., "net-winged") adult insects, are said to be the most sedentary of all arthropod predators, including spiders (Mansell, 1992, 1996, 1999; Topoff, 1977).

To construct its pit, an antlion starts on the surface of the sand, and then moves backwards in a spiraling motion, digging down and flicking sand out of the pit by utilizing its closed mandibles as a shovel (Resh & Cardé, 2009). Once the pit is dug, the antlion positions itself at the center of the conically shaped pit trap, usually just under the surface of the sand with its mandibles open, and waits, sometimes for months at a time without food, for prey to fall inside (Fertin & Casas, 2006; Griffiths, 1980; Matsura, 1987; Scharf & Ovadia, 2006).

Mechanoreceptor setae (hairs), which cover the entire body of a larval antlion (see Figure 3), are exceptionally sensitive to vibrations produced by approaching prey, capable of detecting, as well as localizing, potential prey as far away as 10 cm from the pit edge (Devetak, 1985; Devetak, Mencinger-Vračko, Devetak, Marhl, & Špernjak, 2007; Mencinger, 1998; Mencinger-Vračko & Devetak, 2008). This ability to localize prey at a distance enables the antlion to flick sand in the direction of a potential victim, utilizing its mandibles as a shovel; this frequently observed behavior is thought to disorient prey and increase the likelihood that it stumbles into the pit (Mencinger-Vračko & Devetak, 2008). If the conical shape of the pit does not deliver prey immediately to the

Figure 2. *A photographic montage of pit-digging antlions (Myrmeleon spp.). Close-up view of a pit-building larval antlion exposed on the sand surface (top left) and in the process of burying itself under the sand (top right). Bottom: Typical funnel-shaped antlion pits in sand. The winding furrows on the right side of the photograph are the characteristic tracks made by antlions as they search for a suitable pit location; these tracks give rise to antlions' common name, doodlebugs. Adapted with permission from "Specialized Learning in Antlions (Neuroptera: Myrmeleontidae), Pit-Digging Predators, Shortens Vulnerable Larval Stage," by K. L. Hollis, H. Cogswell, K. Snyder, L. M. Guillette, and E. Nowbahari, 2011, PLoS ONE 6(3): e17958, p.2. Copyright 2011 by Hollis et al.*
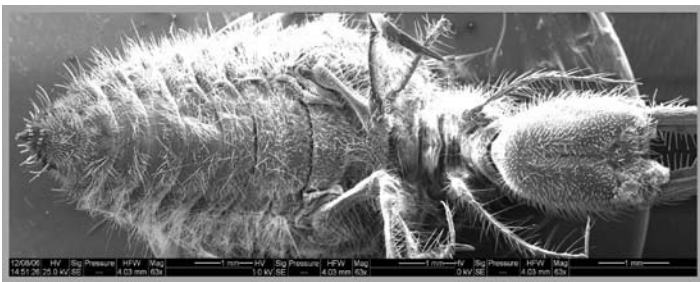
Figure 3. *A montage of three Scanning Electron Microscope (SEM) images of the ventral view of a pit-digging antlion. Mechanoreceptor bristles and hairs can be seen all over the body. All SEMs by Lauren M. Guillette.*

antlion waiting below, it may flick sand at the prey, causing the prey to fall to the bottom of the pit trap (Devetak, et al., 2007; see accompanying videos of antlions capturing prey). When the antlion is done feeding on the prey, it once again

utilizes its mandibles to flick the wasted exoskeleton of the prey out of the pit trap (Mansell, 1999). This prey capture and feeding process disrupts the pit, of course, and thus requires restorative maintenance by the antlion to return the pit trap its original conical shape (Fertin & Casas, 2006).

Antlion Life Cycle

Pit-digging antlions' larval stage, which can last as long as three years (Gotelli, 1993, 1997; Scharf & Ovadia, 2006), is highly variable in length, in large part because it depends on the availability of food (Griffiths, 1980, 1986). During the larval stage, antlions mature through three substages, termed instars, molting between the first and second instar and then again between the second and third instar (Tauber, Tauber & Albuquerque, 2003). To molt, antlions cease feeding and move down into the sand under their pits; when they emerge, they toss their recently shed exoskeletons and resume feeding in the same pit, which increases in size as the antlions themselves grow larger. When third-instar larvae reach a critical mass, they pupate, metamorphosing into reproductively mature, flying adults (Griffiths, 1985). To pupate, larvae again bury themselves in the sand directly under their pits, and then secrete a sticky silk to which fine particles of sand and debris attach (Mansell, 1999). The resulting sandball cocoon offers protection and shelter for approximately thirty days, after which the imago (i.e., adult) emerges. The adult stage, like the egg and pupal stages, is also extremely short, lasting a mere four weeks (Gotelli, 1993). In contrast to feeding behavior during the larval stage, the nocturnally active adults consume little food (Burton & Burton, 1969), their primary function being reproduction. Females lay up to 20 single eggs in multiple locations just under the surface of the sand (Burton & Burton, 1969; Gotelli, 1993; Tauber, Tauber & Albuquerque, 2009), which then hatch into first instar larvae that typically dig pits within one day (Burton & Burton, 1969).

Learning in Antlions

Because pit-digging antlion larvae can be studied easily in the laboratory, they have served as our model species to examine learning abilities and fitness outcomes in the most extreme of sedentary predators. The focus of our research with larval antlions has been to demonstrate that these sit-and-wait predators, like their actively foraging counterparts, would be able to learn associations between environmental cues and prey appearance. Although all sit-and-wait predators typically possess elaborate sensory structures to detect their prey well in advance of its arrival, as described above for antlions, the possibility still exists that some aspects of this predatory response could be modified through learning.

In four separate experiments (Guillette, Hollis & Markari-

an, 2009; Hollis et al., 2011), which we describe in more detail below, individual antlions were fed daily, either receiving a vibrational cue presented immediately before the arrival of food or that same cue presented independently of food arrival. In Experiment 1, we looked for evidence that antlions were capable of anticipating prey arrival through learning. In Experiments 2, 3 and 4, we sought evidence that this learning would provide a selective advantage. In particular, we examined antlions' ability to extract food more efficiently (Experiment 2), which, we predicted, would increase their rate of growth, thereby decreasing the latency to molt (Experiment 3) and, more importantly, the latency to pupate (Experiment 4).

Antlions are able to anticipate prey arrival. In this experiment (Guillette, Hollis & Markarian, 2009, Experiment 1; reviewed in Guillette & Hollis, 2010), antlions received a vibrational cue either immediately before food arrival or at another, randomly determined time of day. The cue was provided in the form of sand falling into a receptacle sitting on the sand surface, and was delivered at one of three different distances, either 3 cm, 8 cm, or 15 cm from the edge of the antlion's pit. Although this particular cue was chosen because it did not elicit any form of observable behavior in pilot work, it is a sensory stimulus that antlions would be certain to detect. That is, antlions' bodies are covered with thousands of hairs and bristles (see Figure 3), each of which contain sensitive mechanoreceptors that respond to vibrations conducted through the sand (Matsura & Takano, 1989). The cue distances that we chose for this experiment are well within the range in which antlions are capable of detecting vibrational stimuli (Devetak, 1985; Devetak, et al., 2007; Mencinger, 1998; Mencinger-Vračko & Devetak, 2008).

Immediately after their arrival in the lab[1], larval antlions, (*Myrmeleon crudelis* Walker) were placed individually in sand-filled plastic bowls. From amongst those that dug pits, pairs of antlions, matched for weight, were created. One member of each pair was randomly assigned to a learning treatment group (LRN); its matched-weight pairmate was assigned to a control treatment group (CTL). Next, each antlion was transferred to a separate, larger sand-filled plastic container (see Figure 4), where subsequent training would take place. This training apparatus was nestled in a sound-and-vibration attenuating chamber made of foam. The vibrational cue, falling sand, was delivered via a lever-controlled plastic dropper, which was fixed above the container housing each antlion, and was caught in another receptacle underneath to prevent additional sand from accumulating near the antlion's pit.

To control for any potential effects of circadian mechanisms on feeding behavior and digestive efficiency, LRN antlions were fed at the same time of day as CTL antlions; a
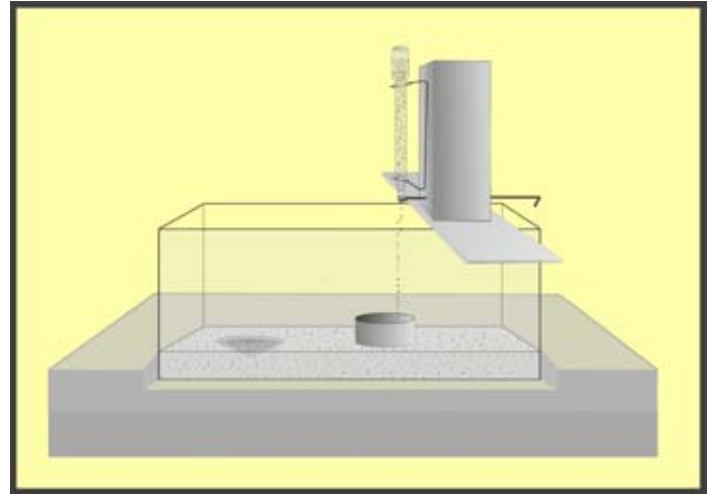

Figure 4. Antlion apparatus. Front view of the apparatus illustrating the rectangular plastic box housing an antlion in its pit. The plastic box is nestled in a foam block; the sand delivery device rests on top of the box, directly over the sand collection container. The sound-and-vibration attenuating chamber (not pictured) surrounds the apparatus on the left, right and rear sides. Adapted with permission from "Learning in a Sedentary Insect Predator: Antlions (Neuroptera: Myrmeleontidae) Anticipate a Long Wait," by L. M. Guillette, K. L. Hollis, and A. Markarian, 2009, Behavioural Processes, 80, p. 226. Copyright 2009 by Elsevier B.V.

single prey item, a wingless fruit fly, was delivered directly to the center of each antlion's pit once each day. However, to prevent time, per se, from serving as a reliable cue for prey arrival, the daily feeding was performed at a randomly selected time each day, between the hours of 10:00am and 5:00pm. For each antlion in the LRN group, the daily feeding was preceded by the vibrational cue (a typical Pavlovian, or classical, conditioning procedure); antlions in the CTL group received the vibrational cue at another, randomly selected time between 10:00am and 5:00pm.

As Figure 5 (top panel) illustrates, the results from this experiment suggest that antlions learned to associate the vibrational cue with food delivery: When the signal was close to the pit (Signal Distance: Near), animals in the LRN group moved their head and mandibles significantly more often than animals in the CTL group. Indeed, a significant increase in LRN animals' response rate was observed after only two training sessions. As Figure 5 also illustrates, however, LRN antlions receiving the signal at the intermediate (Signal Distance: Intermediate) and far (Signal Distance: Far) distances did not exhibit this same anticipatory response (see middle and bottom panels, respectively). The absence of an anticipatory response in LRN antlions when the cue was delivered at either the intermediate or far distance from the pit likely is due to the fact that sand grains often were displaced from
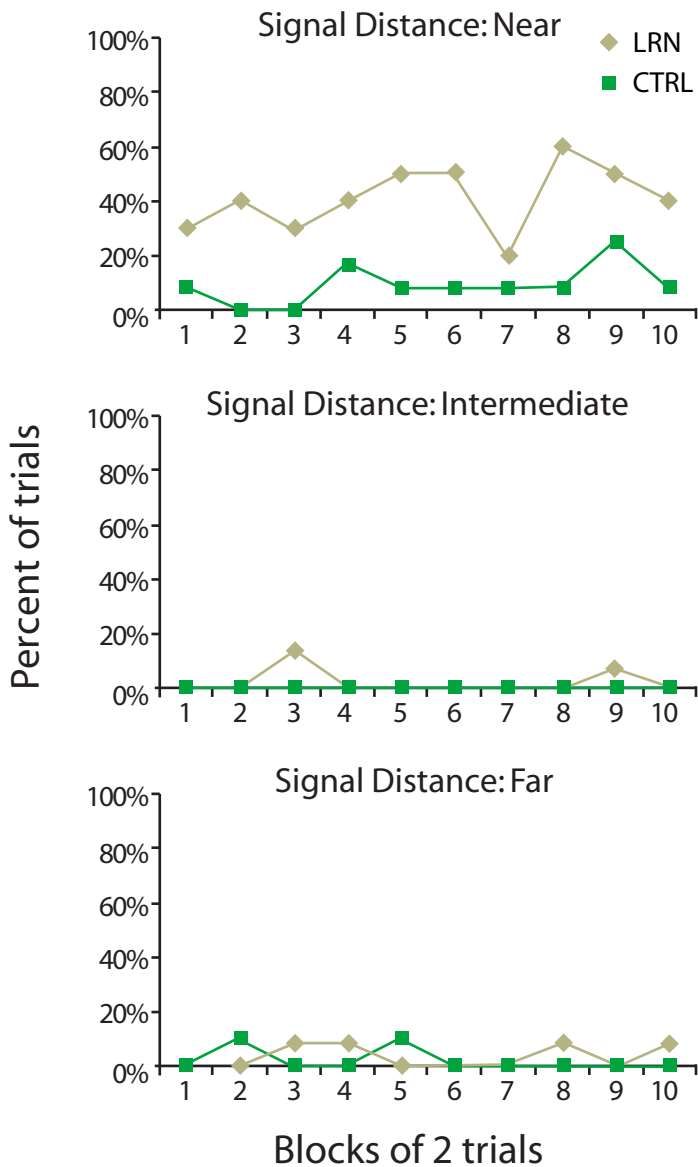
Figure 5. Learned movement response. Percent of trials in which subjects moved in response to a vibrational cue for LRN and CTL subjects in the Signal Distance: Near group (top panel), in the Signal Distance: Intermediate group (middle panel), and in the Signal Distance: Far group (bottom panel) across blocks of 2 training trials. To explore the significant statistical interaction between treatment group and signal distance group, Tukey post hoc tests were conducted, revealing that LRN subjects in the Signal Distance: Near group moved significantly more in response to the vibrational cue than CTL subjects in the Signal Distance: Near group, p < 0.001; however, no differences between LRN and CTL subjects were observed in the Signal Distance: Intermediate or Signal Distance: Far groups. Adapted with permission from "Learning in a Sedentary Insect Predator: Antlions (Neuroptera: Myrmeleontidae) Anticipate a Long Wait," by L. M. Guillette, K. L. Hollis, and A. Markarian, 2009, Behavioural Processes, 80, p. 227. Copyright 2009 by Elsevier B.V.

the sides of the pit only when the vibrational cue was close by, obviously disrupting the pit. Under these circumstances, LRN antlions, but not their CTL pairmates, sought to ready themselves for imminent prey delivery by moving their head and mandibles away from any dislodged sand particles. It is important to note that sand grains were displaced *equally* in both LRN and CTL groups, but only LRN antlions responded during the vibrational signal.

Learning provides fitness benefits: Prey extraction and pit size. Because antlions could, at least potentially, extract food and grow at different rates in each of their three instars, it was important to select subjects for this experiment at exactly the same developmental phase (Guillette, Hollis & Markarian, 2009, Experiment 2; reviewed in Guillette & Hollis, 2010). Thus, prior to their selection as subjects, antlions were fed until they buried themselves under the sand in preparation for molting. When they next re-emerged as third instar larvae, the last instar before pupation, subject pairs were created exactly as in the previous experiment, with one member assigned to the LRN treatment and its matched-weight pairmate assigned to the CTL condition. Training for LRN and CTL subjects also followed the same protocols as described above, except that the vibrational cue was delivered at 4.5 cm from the pit and ants, rather than wingless fruit flies, were used as prey items.

In this experiment, several food extraction measures were calculated each day of training: extraction efficiency (the percent of prey mass extracted), extraction rate (the rate at which animals extracted prey contents), and extraction efficiency rate (the percent of prey mass extracted per unit time). In addition, pit size was measured daily. Although LRN and CTL antlions did not differ in extraction efficiency, the simple percent of prey mass extracted, both extraction rate and extraction efficiency rate were significantly greater in LRN antlions than in CTL antlions (see Figure 6).

The higher extraction rates and extraction efficiency rates exhibited by LRN antlions, compared to CTL antlions, obviously reflect more efficient digestion, which occurs extra-orally in antlions. One possibility is that, at a physiological level, the conditional response to the vibrational cue may have involved anticipatory enzyme production—a response that is similar, of course, to the prototypical classically conditioned salivary response demonstrated by Pavlov (1927), and which in humans and other mammals results in increased caloric extraction (Hollis, 1982), a point to which we will return later. The idea that antlions, too, might have developed a conditional enzymatic response to the cue is not far-fetched: Wantanabe and Mizunami (2005) recently demonstrated anticipatory conditional responses in the salivary neurons of cockroaches. In our experiment, the reason that extraction efficiency, the percent of prey mass extracted, did not differ
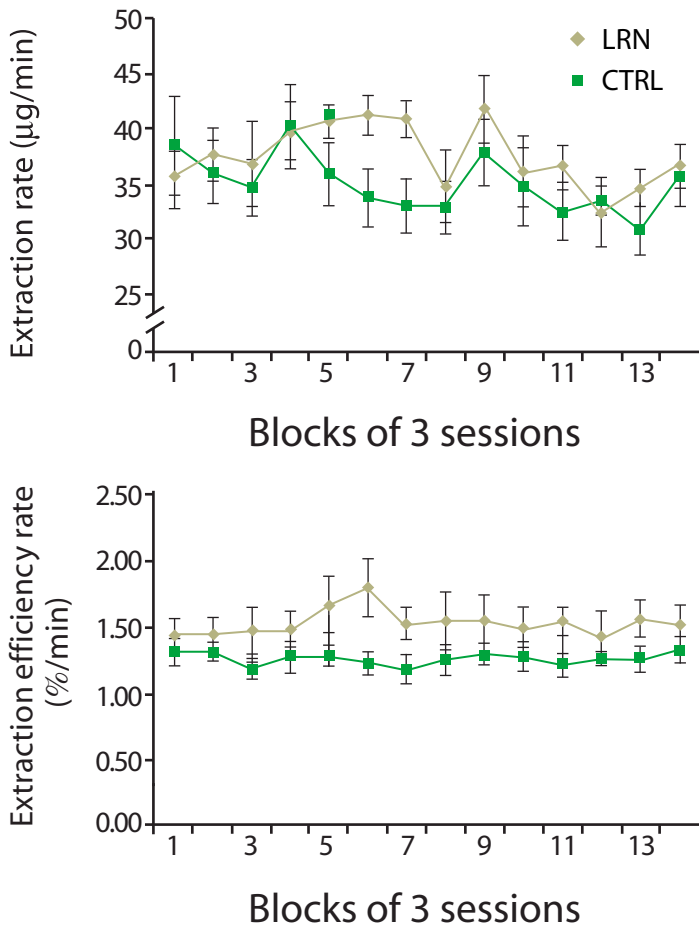
Figure 6. *Extraction measures. Mean ± standard error extraction rate (top panel) and mean ± standard error extraction efficiency rate (bottom panel) of prey consumed by subjects in the LRN and CTL treatment groups across blocks of 3 training sessions. Mann-Whitney U-tests revealed that both extraction rate and extraction efficiency rate were significantly faster in LRN antlions than in CTL animals, $p < 0.05$ and $p < 0.01$, respectively. Adapted with permission from "Learning in a Sedentary Insect Predator: Antlions (Neuroptera: Myrmeleontidae) Anticipate a Long Wait," by L. M. Guillette, K. L. Hollis, and A. Markarian, 2009, Behavioural Processes, 80, p. 229. Copyright 2009 by Elsevier B.V.*

between LRN and CTL antlions is that conditional enzyme production would be certain to have an effect on the two measures of extraction *rate*, but need not affect the amount (or percent) of prey mass extracted. That is, CTL antlions would not have been able to digest their prey as quickly and efficiently as LRN antlions, but would have, given sufficient time, been able to extract the same amount of food mass.

Another reason for the higher extraction rates and extraction efficiency rates exhibited by LRN antlions compared to CTL antlions, may be that LRN subjects simply were more efficient handling their prey. Antlions' mandibles play a significant role in this process, not only in prey capture, but also

in digestion itself. After antlions capture the prey between their mandibles, they use the mandibles to inject a poison, which paralyzes and, subsequently, kills the prey (Yoshida, Sugama, Gotoh, Matsuda, Nishimura & Komai, 1999). Next, they use the mandibles to inject digestive enzymes, which liquefies the prey, and finally to pump the extraorally-digested contents back into the gut (Griffiths, 1982; Van Zyl, Van Der Westhuizen & Van Der Linde, 1998). Thus, any difference in prey handling by LRN and CTL antlions, either during prey capture or during the manipulation of subdued prey, easily would result in extraction rate differences.

Either of these interpretations of our extraction efficiency data—that is, a difference between LRN and CTL antlions in conditional enzymatic release or in handling efficiency—would explain the other main finding of this experiment, namely the comparatively greater increase in pit size exhibited by LRN antlions over the course of the experiment. In addition to extraction measures, pit size also was measured at regular intervals in this experiment and, as Figure 7 shows, antlions in the LRN group were, by the end of training, constructing significantly larger pits than those in the CTL group. Moreover, whereas Figure 7 suggests that CTL antlions reached an asymptote in pit size, LRN animals show no such evidence of nearing asymptotic pit volume.
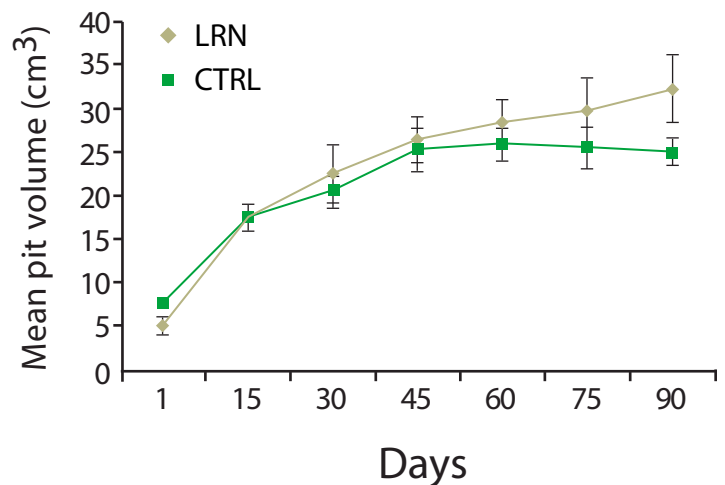

Figure 7. *Mean ± standard error pit volume (cm^3) for subjects in the LRN and CTL treatment groups on training Days 1, 15, 30, 45, 60, 75, and 90. A mixed methods ANOVA, which was used to compare LRN and CTL antlions' pits over days, revealed that pits in both groups increased significantly over days, $p < 0.001$; however, by the end of the experiment, the pits of LRN animals were significantly larger than the pits of CTL animals, $p < 0.05$. Adapted with permission from "Learning in a Sedentary Insect Predator: Antlions (Neuroptera: Myrmeleontidae) Anticipate a Long Wait," by L. M. Guillette, K. L. Hollis, and A. Markarian, 2009, Behavioural Processes, 80, p. 229. Copyright 2009 by Elsevier B.V.*

As a dependent measure, pit volume is important for several reasons. One, larger pits capture larger prey items, which obviously are more energetically profitable (Griffiths, 1986). Two, the larger the pit, the greater the encounter rates with prey (Griffiths, 1980). Indeed, Griffiths demonstrated that as little as a 2 mm increase in pit diameter translated into a 10% increase in capture success. Extrapolating from this result to our own data (see Figure 7), LRN antlions, whose mean pit size was 5.2 mm larger than those of CTL antlions, would under natural conditions be expected to obtain a 25% increase in capture success. Although in our experiment, antlions in both groups captured all prey items dropped into their pits—and, equally importantly, neither latency to capture prey nor latency to discard the prey exoskeleton were different for LRN and CTL animals—this benefit of a larger pit presumably would be realized by antlions in the field.

However, the pit size differences between LRN and CTL groups in this experiment are important for yet another reason: Across multiple replications of data collection in our own lab (see Table 1), pit volume is highly correlated with antlion size, a finding previously demonstrated by other researchers (Day & Zalucki, 2000; Griffiths, 1986). Thus, the comparatively greater increase in the size of LRN antlions' pits, relative to those in the control group, provides indirect evidence that associative learning has a positive influence on growth rate—evidence that is further corroborated by the extraction data, described above. That is, the greater efficiency with which LRN antlions extracted prey contents enabled them to grow at a faster rate, whether that growth came about because of anticipatory enzyme production, which would have enabled LRN antlions to extract more calories from signaled food, or whether it derived from more energy-efficient handling of prey, which would have resulted in a net energy gain, compared to CTL antlions.

Learning provides fitness benefits: Latency to molt. A third experiment explored whether the kinds of fitness benefits demonstrated in the previous experiment, namely larger pit size and more efficient prey extraction, might translate into a faster time to molt (Guillette, Hollis & Markarian, 2009, Experiment 3; reviewed in Guillette & Hollis, 2010).

Table 1. Summary of Intercorrelations Between Antlions' Initial Weight and Initial Pit Measurements

Measure	1	2	3	4	5	6	7
1. Initial weight	—	.576*	.657*	.672*	.706*	.657*	.740*
2. Pit volume - Trial 1		—	.877*	.909*	.867*	.857*	.828*
3. Pit volume - Block 1			—	.822*	.941*	.887*	.933*
4. Pit depth - Trial 1				—	.917*	.789*	.809*
5. Pit depth - Block 1					—	.875*	.921*
6. Pit diameter - Trial 1						—	.960*
7. Pit diameter - Block 1							—

Note: Intercorrelations for antlions (n = 52) across multiple replications of the first two associative learning experiments described in this paper. Initial weight refers to each antlion's weight immediately prior to being moved to its individual training apparatus. Pit measurements were taken following the first day of training (Trial 1), and following the last day of Block 1, which consisted of 3 training trials. Adapted with permission from "Learning in a Sedentary Insect Predator: Antlions (Neuroptera: Myrmeleontidae) Anticipate a Long Wait," by L. M. Guillette, K. L. Hollis, and A. Markarian, 2009, Behavioural Processes, 80, p. 230. Copyright 2009 by Elsevier B.V.
*p < 0.01

In addition, this experiment also was an attempt to replicate the findings of Experiment 2 with antlions in an earlier instar, that is, with second instar antlions instead of third instar antlions. Similar to the previous experiments described above, one group of antlions received the associative learning treatment (LRN), in which a vibrational cue predicted prey delivery, while the other group received a random control treatment (CTL), in which the vibrational cue was presented independently of prey delivery. However, a critical difference between this experiment and the ones described above is that it included a final test day following training: On the day of the test, all animals in both groups received the vibrational cue followed immediately by prey delivery, thus providing a more direct, controlled comparison of the treatment groups. Following the test, LRN and CTL antlions were monitored for an additional seven days for evidence of molting.
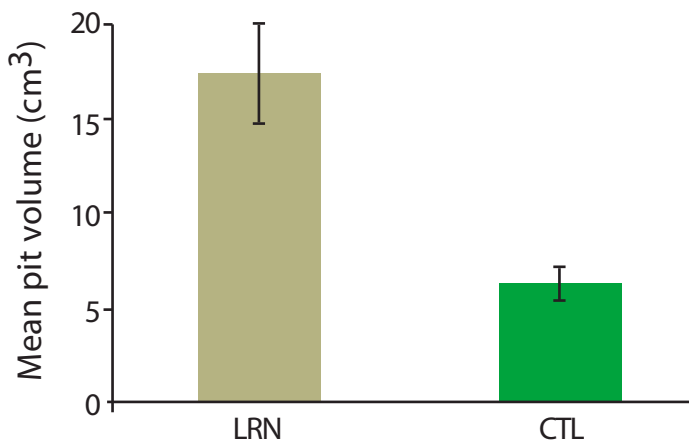
Figure 8. Pit volume on test day. Mean ± standard error pit volume (cm³) for subjects in the LRN and CTL treatment groups on the single test day following training. A Mann-Whitney U-test revealed that the pit volume of LRN antlions was significantly larger than that of CTL animals, p < 0.05.

As Figures 8 and 9 illustrate, on the day of the test, LRN antlions dug significantly larger pits (Figure 8) and exhibited significantly greater extraction efficiency and extraction rate (Figure 9, top and bottom panels, respectively) than CTL animals, thus replicating the results from Experiment 2. Moreover, as Figure 10 illustrates, antlions receiving the LRN treatment molted significantly faster than antlions in the CTL treatment, a finding that not only provides more evidence that the ability to anticipate prey through learning enables antlions to grow at a faster rate than CTL antlions, but also demonstrates another fitness advantage of associative learning. Because antlions remain especially vulnerable to their own predators, for example birds and lizards, throughout their larval stage, any increase in growth rate, and consequent decrease in the length of the larval stage, can significantly decrease generation time and, thus, larval

mortality (Crowley & Linton, 1999), yet another potential fitness advantage of using learned cues to anticipate prey.
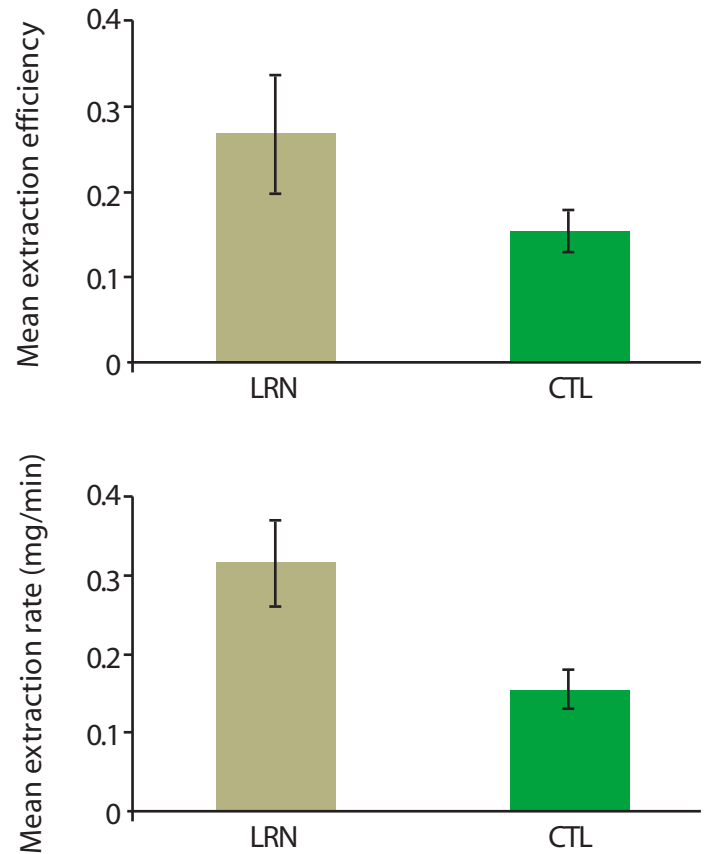

Figure 9. Extraction measures on test day. Mean ± standard error extraction efficiency (top panel) and mean ± standard error extraction rate (bottom panel) of prey contents consumed by subjects in the LRN and CTL treatment groups on the single test day following training. Mann-Whitney U-tests revealed that both extraction efficiency and extraction rate were significantly greater in LRN antlions than in CTL animals, p < 0.05 for both measures.

Learning provides fitness benefits: Latency to pupate. A fourth experiment, recently published (Hollis et al., 2011), explored whether the increase in growth rate demonstrated in Experiment 3 might also decrease the latency to pupate, thus providing significantly more powerful evidence that the ability to learn could decrease generation time and, thus, larval mortality. This experiment was similar procedurally to the one described above in which Guillette, Hollis & Markarian (2009) waited for pairs of antlions to reach their third instar, and then assigned one member of each pair to the associative learning treatment, in which a vibratory cue preceded prey delivery, and its pairmate to the random control treatment, in which the vibratory cue and prey delivery were not paired. However, in this case, Hollis et al. (2011) trained animals for six days each week, continuing each subject's training either until it pupated, or until the end of the experiment on Day 70.
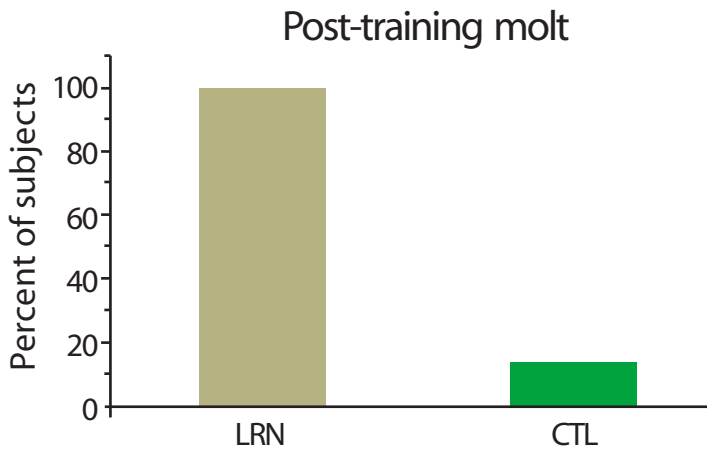
Figure 10. Percent of subjects in the LRN and CTL treatment groups molting by Day 24. A Chi-square analysis revealed that LRN antlions molted significantly sooner than CTL antlions, p < 0.01. Adapted with permission from "Learning in a Sedentary Insect Predator: Antlions (Neuroptera: Myrmeleontidae) Anticipate a Long Wait," by L. M. Guillette, K. L. Hollis, and A. Markarian, 2009, Behavioural Processes, 80, p. 231. Copyright 2009 by Elsevier B.V.

A biostatistical technique, called Kaplan-Meier survival analysis, was used to compare the rate at which subjects in the learning and control groups pupated: As Figure 11 illustrates, subjects receiving the associative learning treatment not only began dropping out of the experiment (pupating) as early as Day 35, a full week before the first control subject pupated on Day 42, but also the significantly steeper slope of learning animals' survival curve indicates that associatively trained antlions pupated significantly faster than did control subjects. Median survival time, an often-used survival statistic that corresponds to the point at which half of the subjects remain in a given treatment, was 46 days for antlions in the learning group; this same point was not reached, even by the end of the experiment on Day 70, in control antlions.

Learning in antlions: A summary. When taken together, the four experiments reviewed here demonstrate that antlions, extremely sedentary, sit-and-wait predators that do not engage in any form of active search for their food—and that already are equipped to anticipate prey arrival via a very elaborate sensory detection system—nonetheless are capable of associative learning. Moreover, these experiments demonstrate that antlions' capacity for associative learning greatly enhances prey capture and, in turn, provides them with several potential fitness advantages. When, in Experiment 1, an environmental signal predicted the appearance of a potential prey item, antlions exhibited a learned response, namely a movement that freed their head and mandibles of dislodged sand, allowing them to be ready for the imminent arrival of prey. Although this particular learned response
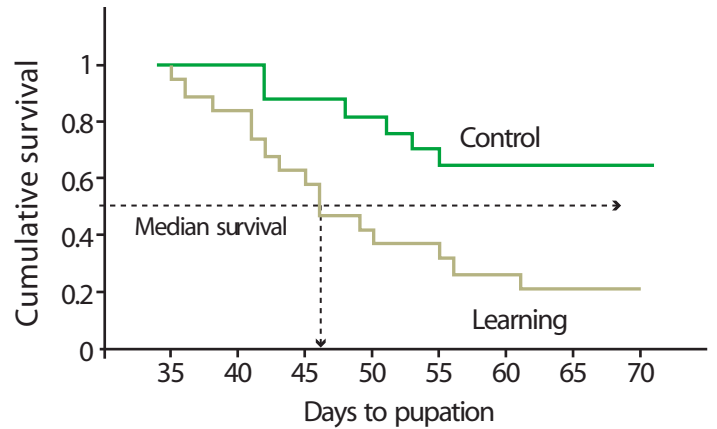

Figure 11. Kaplan-Meier survival curves illustrating days to pupation in matched pairs of Learning and Control subjects (n = 36). Following 70 days of treatment, 79% of Learning antlions pupated (15 of 19), while only 35% of Control antlions pupated (6 of 17). Median survival time, here median days to pupation, corresponding to the time point at which half of the animals remained (i.e., 50% cumulative survival), was 46 days for Learning antlions; median survival time was not reached in Control animals, even by the end of the experiment on Day 70. Adapted with permission from "Specialized Learning in Antlions (Neuroptera: Myrmeleontidae), Pit-Digging Predators, Shortens Vulnerable Larval Stage," by K. L. Hollis, H. Cogswell, K. Snyder, L. M. Guillette, and E. Nowbahari, 2011, PLoS ONE 6(3): e17958, p.3. Copyright 2011 by Hollis et al.

may have been specific to the experimental preparation, the ability to associate a learned cue with prey arrival enabled antlions, in Experiment 2, to extract the contents of their prey more efficiently than animals not given the opportunity to pair the cue with food. In turn, this enhanced capacity to extract prey contents resulted in faster larval growth, as reflected in increased pit volume. Finally, both supporting and expanding the interpretation of faster larval growth, Experiments 3 and 4 demonstrated that the capacity for associative learning enabled antlions to molt—and, more importantly, to pupate—sooner than animals without this additional, learned ability to anticipate prey arrival.

Although the vibrational cue used in these experiments might seem unnatural at first, in fact it may not be all that different from naturally occurring prey capture. In short, if antlions are able to detect substrate-borne vibrations at long range, that is at distances longer than those that elicit observable motor behavior (e.g., Devetak, 1985; Devetak, et al., 2007; Mencinger, 1998; Mencinger-Vračko & Devetak, 2008), then they may be able to use these cues to further ready themselves for prey capture. That is, vibrations generated by prey while still too far away to make sand-tossing effective, nonetheless might serve to ready antlions for a po-

tential capture attempt as the prey moves closer, and prepare them in other ways, such as those described above.

Conclusions

The idea that learning has evolved to help animals search in an unpredictable environment for food, hosts or mates certainly is not new. No sooner did the "constraints on learning" literature (Hinde & Stevenson-Hinde, 1973; Shettleworth, 1972) inspire researchers to think about the adaptive function of learning, than they began to theorize about the value of using learned cues to aid that search (Hollis, 1982; Moore, 1973; Staddon, 1983). Although this kind of theorizing was at first limited to vertebrates—notably because insect learning was still in its infancy—it would have been easy to assume that, if *some* species of insects could learn, they were most likely species that also needed to search their environments. And, from there, it would have been another easy theoretical step to assume that active search and avoidance behavior could be an important indicator of which insect species would be expected to possess the capacity for learning (e.g., Bernays, 1993). Who would have predicted, even in the early-1990s, that in the next two decades associative learning would have been documented in so many different species of insects, which, taken together, represent most major insect orders (see reviews by Dukas, 2008; North & Greenspan, 2007; Papaj, 2003; Papaj & Lewis, 1993).

No doubt inspired by the ever-growing list of insects capable of learning, Dukas (2008) has expressed the view that learning may be universal in all animals with a nervous system. Even stronger, more expansive assertions have been made by neuroscientists and others who study brain architecture, namely that learning emerges as a "fundamental principle of brain functionality" (Greenspan, 2007, p. 649). Moreover, recent research in the field of systems biology not only suggests what would have been an astounding assertion only a few years ago, but also highlights how quickly neuroscientific assumptions themselves are likely to change in the years ahead: Some researchers (e.g., Fernando, et al., 2009) have argued that associative learning may not even be "confined to neural systems (p. 463)." That is, single celled organisms, as well as molecular circuits *within* organisms, may exhibit fundamental properties of associative learning.

In the context of assertions that learning is an emergent property of neural circuitry, and an ever-expanding list of organisms capable of learning, providing more and more evidence for this view, how do we make sense, then, of theories and models that attempt to explain or predict the evolution of learning, implying, as they do, that some species, even those with nervous systems, do not possess that capacity? Although the thrust of what we call the "environmental unpredictability" models is the evolution of learning *qua* learning,

one possibility is that these models actually are exploring the conditions under which plasticity is maintained or overridden, just the reverse of what they currently posit. That is, although models such as Dunlap and Stephen's (2009) demonstrate quite convincingly how different kinds of environmental predictability produce the capacity for inflexible vs. learned behavior, how can we be certain which of these phenotypes is the default condition? Of course, the view of learning as an emergent property of neural circuits does not change Dunlap and Stephen's conclusion that "some types of environment change favor learning while others select against it (p. 3201)." Rather, what changes—and we argue that this change is critical to reconciling "environmental unpredictability" models with neuroscientific findings—is the current emphasis on the "evolution of *learning*" instead of an emphasis on the "evolution of behavioral *inflexibility*."

Other work in the field of insect behavior also is at odds with the view that learning is an emergent property of neural circuitry, namely research that explores what are thought to be the "costs of learning" (see Burns, Foucaud & Mery, 2010, for a review). That is, the ability to learn supposedly carries costs, called constitutive (or global) costs that result from the development and maintenance of the requisite neural structures involved. Thus, this costly trait should emerge only when the price is worth paying. However, as Snell-Rood and Papaj (2009) have demonstrated in cabbage white butterflies, *Pieris rapae*, the costs of maintaining behavioral plasticity, in this case the ability to learn about host-plants, may be sufficiently low that, even in a highly predictable environment in which inflexible innate behavioral patterns are favored, the ability to learn could be maintained. However, it's still difficult to reconcile the "learning-as-an-emergent-property" view with one that argues that associative learning involves costs of any kind—despite the fact that much solid research supports the latter (Raine, 2009; Snell-Rood & Papaj, 2009; see Burns, Foucaud & Mery, 2010, for a recent review).

One answer to those who would suggest that associative learning carries costs, might lie in the paradox described by Raine (2010): If, as he suggests, learning requires little more than "a sense organ and a simple neural circuit with a switch (which can be reinforced)," then, we agree, it's very hard to understand why the kind of elaborate brain architecture that is typical of insects' mushroom bodies increases so dramatically with experience. The answer, perhaps, is that what we are looking at in the architecture of mushroom bodies is far, far more sophisticated than the simple ability to form learned associations. That is, the "costs of learning" are not costs of simple associative learning, per se, which emerges *ipso facto* from simple neural circuitry. Rather, they are costs associated with a panoply of cognitive skills that far surpasses simple associative learning abilities. Similarly, and here we

return to Farris and Schulmeister's (2010) argument that the associative learning demands of parasitoidism drove mushroom body expansion, we suggest that it's not the capacity for associative learning that requires so much elaborate mushroom body circuitry, or, perhaps, even the number of different associations that insects can learn, but rather much more advanced cognitive feats such as those that are just beginning to be explored in insects, such as numerosity (Chittka & Geiger, 1995; Dacke & Srinivasan, 2008) and concept formation (e.g., Avarguès-Weber, Dyer, & Giurfa, 2011; Giurfa, Zhang, Jenett, Menzel & Srinivasan, 2001)—and likely many others that still await discovery.

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