

The ability of ants to associatively learn based on olfactory chemical cues produced by plants.

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Abstract:

Associatively learning is commonly observed throughout the natural world in many interactions between species and their environment. We know ants utilize environmental cues in their foraging patterns to locate and recruit to food, but little is known about their ability to associatively learn to forage using these cues. Associative learning in ants has been tested in laboratory settings but the experiment has never been replicated in the field. It is also unknown to what extent the ants learn on certain environmental cues including olfactory cues. After allowing ants to forage and recruit to baits associated with scents produced by plants associated with sugar rich resources (either *Ligusticum porteri* or *Helianthella quinquenervis*), we found that additional baits with the same scent were significantly more likely to be discovered than those with the other scent (30-40% more likely to be discovered), although there was no significant difference in how quickly the baits were discovered or the number of ants that recruited to the bait. Furthermore, significantly more ants recruited to the baits if the first bait smelled like *Ligusticum porteri* as opposed to *Helianthella quinquenervis* (23% more likely). This result indicates that ants detect plant scents when foraging for sugar rich resources and subsequently forage more frequently for resources on those same plant species. This learned association could increase the capability of ants to forage successfully and increase the fitness of ant colonies.

Introduction:

The process by which animals associatively learn to forage on resources in more favorable microhabitats is a well observed phenomenon in nature. Associative learning is defined as learned responses to a positive reward such as resources (Pearce 2001). For example, prey species pick up on environmental “alarm” cues that can reduce their foraging activity (Maerz 1998). They are able to associate the “alarm” cues with the presence of a predator and therefore reduce their foraging activity to avoid risking their survival. Another example of animals utilizing associative learning comes in the form of “prey switching”, where predators learn to preferentially feed on prey species when they become more abundant (Murdoch 1969). Furthermore, plants release volatile chemicals when in response to herbivory that act as signals to species of predators to patrol the plant for unwanted herbivores (Agrawal 1998). The use of Herbivore induced plant volatile (HIPV's) is common in several insect-plant interactions in which herbivores will feed on plants and the plants will release VOC's (Volatile organic compounds) in order to attract predators of the herbivores attacking the plant (Kessler 2001). The release of these VOC's then acts as an environmental cue for a positive reward for the ant species in which they learn to associate the VOC elicited by the plant with the presence of prey. Thus, we know that animals have an ability to adjust their foraging behavior in response to cues detected in the microhabitats that affect their foraging patterns, but it is unclear whether this ability has a significant role in the organism's fitness and truly influences the chances of survival in the organism.

Ants have widespread ecological effects in communities through their foraging for plant-associated resources (Styrsky and Eubanks 2007), but it is unknown whether ants can

associatively learn when foraging for these resources and what the broader consequences of these associations are. Ant protection mutualisms occur when ants protect plants or hemipterans from natural enemies (i.e., herbivores or predators and parasitoids, respectively) in exchange for a sugary reward (e.g., extra floral nectar or honeydew, respectively) which are important components of ant diets (Lach 2010). Through these interactions, ants can drive the abundance, distribution, and the evolution of these plants and herbivores (Blüthgen 2000). Ants are known to develop learned foraging behaviors through cues but the idea that ants can recognize cues as identification for species of plants associated with resource availability is something that is quite unexplored. The idea has been tested in lab settings but has yet to be continually explored in a natural setting where a number of variables may come into play. This experiment seeks to evaluate whether ants use olfactory environmental cues in regards to resource foraging in nature.

Ants are known to learn in response to environmental cues such as chemical, olfactory, and visual cues (Aron 1988), which may allow ants to recruit to a food source more efficiently. For example, the desert species of ant, *Cataglyphis bicolor*, have found that these ants can locate a food source up to thirty meters from the mound using olfactory cues carried by wind to the source (Wolf 2000, Steck 2009). Furthermore, the ants *Camponotus fellah* and *Camponotus aethiops* has been found to detect olfactory cues and associate these cues with a sugar-rich reward, subsequently choosing these cues in future interactions when foraging for resources (Dupuy 2006). The same species has been observed to associate another olfactory cue with a negative response (Guerreri 2010). Another species of ants, *Formica fusca* have also been found to utilize spatial associations in which they can construct a mental map using visual cues to find either food resources or to find their way between the colony and the foraging resource (Cosens 1985). This use of learned associations, olfactory cues and other learned behaviors tend the idea

that species of ants have the ability to use associative learning to develop more successful foraging techniques to better allocate resources through the presence of environmental cues.

Thus, foraging ants may detect and associate cues such as color, smell or texture to recognize a host plant species as a provider of sugar rich resources (e.g. extra floral nectar or honeydew). Ants may use environmental cues to locate honeydew secreted from aphid mutualists or extra-floral nectar secreted from the host plant (Lach 2010). There has been found to be positive correlation between the honeydew flicked off a plant onto the ground below as a result of aphid feeding and the intensity of ant presence among the same plant being tended by the aphids (Del-Claro 1996). This exhibits that ants will find honeydew when foraging and then begin their ascent to the top of the plant to search for the colonies of aphids from which the honeydew came. This discovery begs the question that ants may associate the cues presented upon discovery of the honeydew and learn to recognize the species which hosted the aphid colony.

The question then remains, do ants recognize plant cues and learn to associate these cues with greater resource availability and are these associations innate? We address the question using the *Ligusticum porteri*, which hosts the ant-tended aphid *Aphis helianthi* (Apiaceae), and *Helianthella quinquenervis* (Asteraceae) that produces extra-floral nectar (EFN). The work will also give us a greater insight into the mechanisms upon which ants forage and whether or not these mechanisms are innate or learned. We hypothesized that ants use olfactory cues to recognize and learn to preferentially forage on species of plants associated with sugar rich resources (e.g. EFN or honeydew) in previous interactions. We predicted that ants would use associative learning to recognize these plant species as favorable species to forage upon when presented in later interactions.

Materials & Methods:

Natural History:

The research was conducted in the East River Valley near Gothic, CO (38°57'54.7"N 106°59'36.2"W, 38°57'58.5"N 106°59'34.1"W, 38°57'37.1"N 106°59'00.2"W) from June 16th, 2017 to August 4th, 2017. The research took place in three sites that consisted of two 50-100 m transects. The transects were separated by a minimum of ten meters, to avoid non-independence between transects.

The two species of plants that were used include *Ligusticum porteri* which is a species found in the Apiaceae family, and commonly a host of aphids. The other species is *Helianthella quinquenervis* which is a member of the Astaracea family, and a common producer of EFN's. The three species of ants found in the experiment were *Formica fusca*, *Tapinoma sellis* and *Formica rufa*.

Determination of ant response to the scent of two plant species:

To test whether ants can associatively learn based on the scents of the two plant species, we recorded ant foraging activity at experimental sugar baits associated with scents from either *Ligusticum porteri* or *Helianthella quinquenervis* deployed every 10 m along a transect. Sugar baits were attached to a 10 cm bamboo stick and consist of 10 mL of 25% honey solution by mass in a 15 mL centrifuge tube. I rubbed crushed leaves of one plant species (*Ligusticum porteri* or *Helianthella quinquenervis*) on each of the bamboo sticks, alternating between species at each location along the transects. 4-5 days later we repeated the experiment with the other plant species scent in the same location. Ants were allowed 24 to 72 hours (depending on ant

activity) to recruit from the baits, after which we recorded the number of ants that were feeding at the bait. Once ants recruited to the bait, two additional baits were placed approximately 40 cm from the initial bait. We recorded the number and identity of ants present at 30, 45, and 60 minute intervals. The initial bait was then removed and the two sugar baits were left out for a 2-3 hours and the numbers of ants present were recorded one additional time.

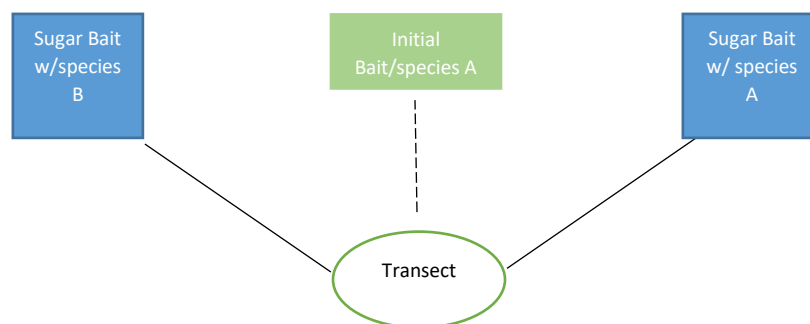


Figure 2. The figure above represents the experiment setup that will sequel the first setup mentioned above. In this setup, the initial learned bait with species scent (green) will be introduced and later followed by two sugar baits (learned species and control).

Analysis of data

All analyses were conducted in R.3.3.4 (RCore Team 2017). The data was analyzed with linear regression models and generalized linear models. We tested whether the bait discovery by ants depended on the main effects of initial and secondary bait scents and their interaction using a generalized linear mixed model with bait set included as a random effect and a binomial distribution. For this analysis, we used the “glmmadmb” function in the “glmmADMB” package (Fournier et. al 2008). For the baits that were discovered, we tested whether the time required for the ants to discover the baits and the number of ants that recruited to the baits depended on the

main effects of the initial and secondary bait scents and their interaction using two separate linear models. For linear models we used “lm” function in the “stats” package (R Core Team 2017) and we conducted F-tests and obtained p-values for all models using the “Anova” function in the “car” package (Fox 2011).

Results:

We found that whether a bait was discovered significantly depended on the interaction between the scent of the initial bait and the scent on the secondary bait ($F_{1, 65} = 5.4347$, $P = .02285$) and the main effect of the initial bait scent ($F_{1, 65} = 4.2730$, $P = .04271$) but not the main effect of the secondary species ($F_{1, 65} = 2.2668$, $P = .13701$) (Figure 1). Of the baits discovered by ants we found no significant effect of the interaction between the initial bait species and the secondary bait species on the amount of time before ants discovered the baits ($F_{1, 40} = .04388$, $P = .512$) and there were no main effects of the initial bait species ($F_{1, 41} = .0452$, $P = .8326$) or the secondary bait species ($F_{1, 41} = .0025$, $P = .9607$) (Figure 2). Furthermore, the number of ants that recruited to the baits did not depend of the interaction between the initial species scent and the secondary species scent ($F_{1, 40} = 1.9119$, $P = .17442$) and there was no main effect of the secondary bait species ($F_{1, 41} = .2110$, $P = .64839$). However, there was a marginally significant main effect of the initial bait scent ($F_{1, 41} = 3.6812$, $P = .06201$) with more ants recruiting to the baits that were added to the initial bait with a *Ligusticum* scent (Figure 3).

Discussion:

We found that there was a significance in ant discovery of the secondary bait based on the interaction between the initial bait scent and the scent of the secondary bait, indicating that

ants use olfactory cues from plants to associatively learn to forage with greater success. Based on the results, it appears ants were more likely to recognize the scent that was the same as the scent of the initial bait when the secondary baits were deployed and were more likely to recruit to that bait. This significance was indicated in trials of both species scents and did not appear to favor one species over the other, although there was a slight significance for *Ligusticum porteri* when we tested how many ants recruited to the baits, of those that were discovered. This result was only significant in the case of *Ligusticum porteri* being the initial bait, which was recruited to by a greater amount as opposed to *Helianthella quinquenervis*, but once the secondary baits were placed out, there was no significance in the number of ants that recruited to each bait.

Discovery of the secondary baits is a product of associative learning and the ant's ability to recognize environmental cues in foraging strategy. We know from previous studies that ants can associate a positive reward with a certain cue in lab settings (Dupuy 2006, Cosens 1985). . In previous lab experiments, the ants were easily able to associate positive rewards because variables in the natural environment were negated. From this study, we now know that ants can associate with these variables in effect and can use natural cues to associatively learn. The ability of ants to then use these associations may lead to an increase foraging success and the development of strategies around such environmental cues. Associative learning could then be used as a tool that enhances ant colony fitness.

The significance in the number of ants that recruited to the initial bait as *Ligusticum porteri* as opposed to *Helianthella quinquenervis* may be a product of a number of differences between the two species. The first is the fact that *Ligusticum porteri* possesses a stronger scent than *Helianthella quinquenervis* and therefore may be overall more attractive to the ants found in the general area. Another reason that could be indicative of why ants discover *Ligusticum porteri*

at a higher rate than *Helianthella quinquenervis* could occur in response to the resource availability of the two species. *Ligusticum porteri* hosts aphid that can occur in large numbers (up to >3,000 aphids per plant) and therefore may be a greater resource for ants as opposed to *Helianthella quinquenervis*, which only produces EFN's. With the information that ants can associate environmental cues with a certain resource, we then offer the idea that ants may be able to distinguish the two species from one another and possess a stronger attraction to the one that they have learned to associate with a more productive resource.

Ant's ability to associatively learn may increase individual and colony fitness as well as playing a significant role in the ecosystem they inhabit. Through the creation of more successful foraging habits by using these environmental associations, individuals could greatly increase the colony fitness by becoming better at discovering resources and allocating less time and energy to finding these resources. If an organism allocates less energy to foraging, it can then distribute this energy to other processes vital to survival in its life. Ants that possess the ability to learn associatively then become more efficient foragers in the population and may then increase their fitness within a colony, thus over time driving the colony as a whole to possess the ability to recognize environmental cues and learn associatively. Furthermore, ants play a large role in the whole system in the form of bottom up processing (ants as an organism affect the trophic levels above them) and the ability of ants to recognize plant associated resources when foraging may affect the fitness of other organism's. In the case of *Ligusticum porteri*, ants are known to tend aphids (Mooney 2008) in which the aphids appear to be detrimental to the plants fitness. Ants also may become more aggressive based on their interactions with large colonies of aphids leading to their predation on herbivores they would more often leave alone (Way 1963). An increase in ants tending aphids could decrease herbivore abundance by almost 28% found in a

study on wood ant species (Fowler and MacGarvin 1985). All these factors reflect the bottom up processing that could be altered based on the ability of species of ants to associatively learn positive rewards by way of olfactory cues and extend their foraging success.

We have seen in lab settings in a number of other studies that species of ants can develop associations (Dupuy 2006, Wolf 2009, Steck 2000, Guerreri 2010, Cosens 1985). This study was the first to illustrate their ability to associatively learn through environmental cues. This study should give way to a number of other studies in which we can develop experiments to single out the cues in which ants can develop associations including other possible cues such as texture, visual, and chemical cues (i.e. VOC's, HIPV's, etc.). It also would be informative to determine if ant species differ in their abilities to develop learned associations. The results from this study indicate ants associatively learn, but another future study could tackle the question of why ants associate certain cues in regards to others or even why they associate cues with certain species as opposed to others. We could then understand how the mechanisms work in the natural world and by which priority ants take in using certain cues. Are ants more likely to associate olfactory cues as opposed to visual cues? Is there an environmental context at play in which ants will choose a certain species over another based on resources or availability? All these questions could serve as future experiments branching from the experiment that we conducted.

In summary, the experiment showed evidence that ants can associate environmental cues with positive rewards and use these associations to discover resources faster in later interactions with the species and the cues. Further research is needed to evaluate the contexts in which ants associatively learn in response to environmental cues, the mechanisms by which they associatively learn, and the consequences for ant foraging success. To determine the

consequences of learned associations, more studies must be conducted in the natural setting that will continue to develop our greater understanding of the ability of ants to associatively learn.

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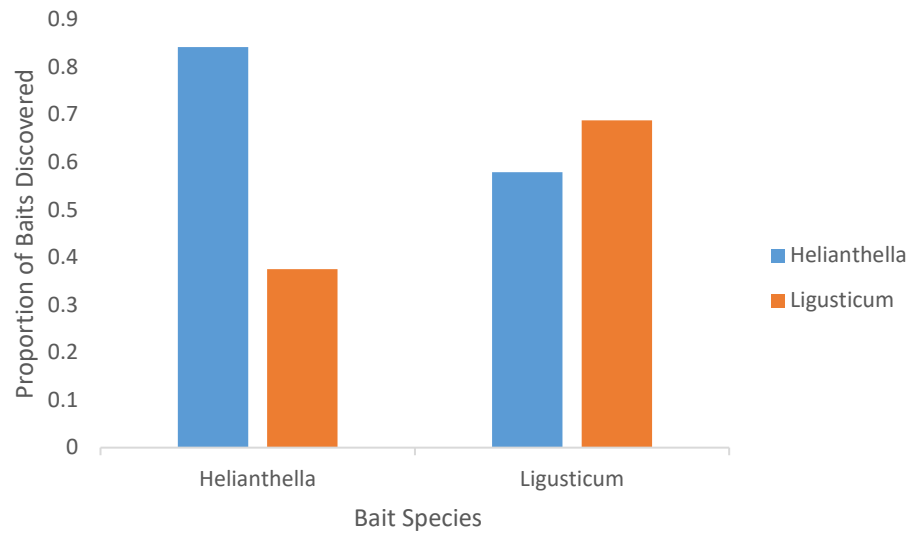


Figure 1.1. The proportion of baits discovered based on the interaction between the first initial species scent and the secondary bait species ($F_{1,65}=5.4347$, $P=.02285$) and main effect of initial bait scent ($P=.04271$) but not the main effect of the secondary bait scent ($P=.13701$)

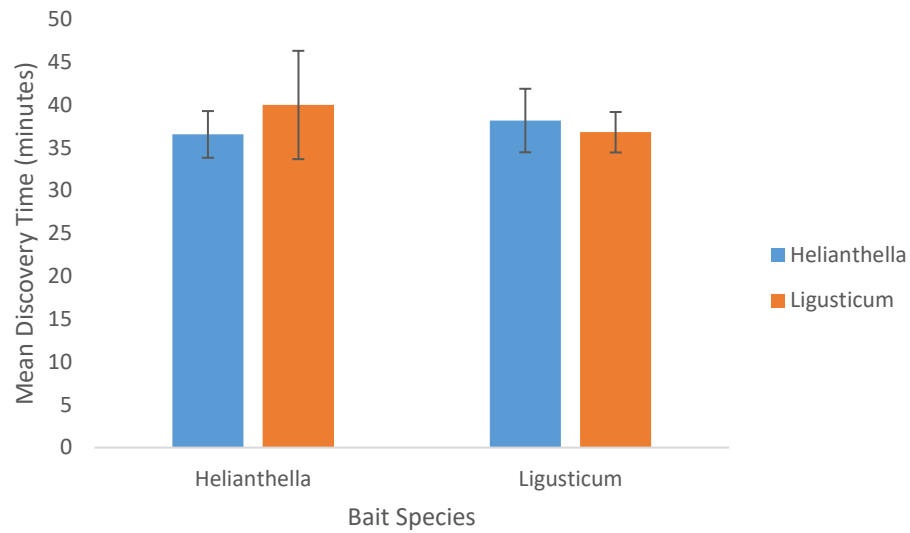


Figure 2. The mean discovery time of the secondary baits in interaction with the initial bait ($F_{1, 40} = .04388$ $P=.512$), no main effects of initial bait species ($P=.8326$) or secondary bait species ($P=.9607$)

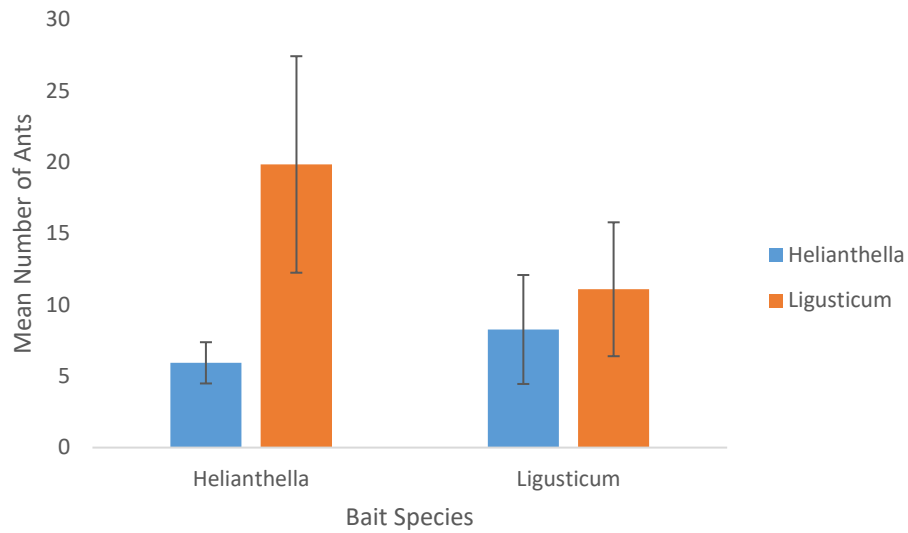
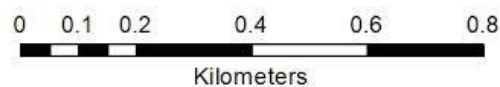


Figure 3. The mean number of ants that recruited to the baits did not depend on the interaction between the first initial bait scent and the secondary bait species ($F_{1,40} = 1.9119$, $P = .17442$) but there was slight significance of main effect of the initial bait scent ($P = .06201$)

Location of Transects within East River Valley



Projection: NAD 1983
Credits: RMBL

Keegan Sentner
8/13/17

