

Are ants botanists?:
Ant associative learning of plant volatiles

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Abstract:

Ants are important members of most terrestrial ecosystems, and often forage in plant canopies where they occasionally participate in mutualisms with either the plants, or aphids that live on the plants. To participate in these mutualisms, the ants must first find them, and it is not clear how ants do this while foraging in the field. It is known that while ants forage in plant canopies they encounter many plant volatiles. There is also some laboratory evidence of associative learning in ants. This leads to the hypothesis that the ants may be learning to recognize the scents of plants that host resources, and preferentially forage on similar smelling plants. In this study we explore whether ants exhibit an association between the scent of a plant, and a honey reward, and whether this is dependent on the local plant density, and thus scent density, in the surrounding area. The two plants used in the study were *Helianthella quinquenervis*, an ant mutualist, and *Ligusticum porteri* a host for aphids, another ant mutualist, allowing us to better understand how this relationship may affect the plants as well. We found that ant foraging may be affected by both the surrounding density of resources, and associative learning. This is important as it brings us closer to understanding how ants interact with their environments across trophic levels, as well as how they and their mutualists may be affected by changes in the environment.

Introduction:

Ants are dominant members of most terrestrial ecosystems, where they occupy multiple trophic levels and roles. Their nests can redistribute nutrients in the soil or aerate the soil through their construction of deep tunnels and chambers (Dostál et al. 2005). These chambers can add nitrogen to the surrounding soil, as the garbage chambers of leaf cutting ants do (Verchot 2003). Qualities that can be important to the health of the surrounding soil. Ants also disperse the seeds of plants, either by bringing seeds to their nest to feed on pieces not necessary for germination, or by disposing of them in areas where they can sprout (Hanazawa et al. 1988). Additionally, they predate on herbivores or parasites of plants that would otherwise harm plants (Bartlett 1961). This protection of plants extends to one last important way they participate in their ecosystems: their mutualisms. Ant mutualisms involve the ants protecting hemipterans or particular plants against natural enemies in exchange for sugar-rich resources (e.g., honeydew or extrafloral nectar, respectively) (Bronstein 1998). What we don't know is how ants are finding these resources in the field: if they are deciding where to forage, and, if they are deciding, how they decide.

Much of ant foraging occurs in plant canopies, where ants collect both floral and extrafloral nectar, prey on other arthropods, and consume honeydew (from aphids and lycaenids) (Mooney et al. 2016). This creates opportunities for associative learning using plant volatiles in countered in the canopy to find critical resources. Associative learning is related to classical conditioning as described by Pavlov (1927), whereby providing a stimulus, such as the ringing of a bell, at the time of offering a reward results in an association between the stimulus and the reward. Thus it is possible that the ants could be using associative learning to construct a chemical search image while foraging.

This leads to an aspect of this ant foraging that is unclear: To what extent are ants using associative learning when they forage if at all? In ants there is evidence of association between chemical stimuli and behavior. Workers of the species *Acromyrmex lundii* can be conditioned by the odor of food fragments brought back by other workers, and preferably forage for that food (Roces 1993). *Atta colombica* ants, which cultivate fungal gardens, can learn to recognize odors that help them avoid gathering plants that are harmful to their mutualistic fungus, even remembering this information up to 18 weeks later (Saverschek et al 2010). Lab experiments have shown that *Camponotus* ants can learn to associate scents with both positive and negative reinforcement (Dupuy et al. 2006). At the same time these associations may be context dependent. Members of the species *Camponotus aethiops* continued to treat non-nestmates aggressively even after the scent of the non-nestmates had been successfully associated with food (Bos, Guerrieri, & d'Ettorre 2010). Thus, although there is evidence for associative learning and recall, it is unclear what the ants are capable of learning, for instance ant scents versus plant volatiles, as well as to what extent and in which contexts the learning influences ant foraging decisions, particularly in a field setting.

In the field plant population density will differ from meadow to meadow (observed.) As such, the availability of possible mutualisms for ants will also differ in density from meadow to meadow. To acquire enough resources, ants would need some way to adapt to this natural variation. If associative learning is this method, then there is a possibility that ant foraging would be dependent on the density of local plant resources.

In this study, we used experimental baits to test whether ants exhibit associative learning while foraging for plant associated resources. Specifically we asked:

- 1) Do ants form positive associations with volatiles produced by the plants *Ligusticum porteri* and *Helianthella quinquenervis*? Plants that host aphids, and provide extrafloral nectar respectively.
- 2) Is ant associative learning dependent on plant density and the availability of plant-associated resources?

By answering these questions we will better understand the mechanisms behind how ants obtain resources, what effects those mechanisms will have on organisms or ecosystems that rely on them, and how environmental changes might affect the ants and their foraging.

Methods:

Study sites

Our study was conducted in eight sites in open, rocky meadows near the Rocky Mountain Biological Laboratory in Gunnison County, Colorado, USA. Five sites were located near the Judd Falls Trailhead or along the 401, and three sites were located near the Snodgrass Trailhead. (fig. 1).

Study species

We cataloged every ant species that we encountered in our experiment, but focused on the two most abundant species: *Formica fusca* and *Tapinoma sessile*. Other ants observed were *Formica rufa*, *Lasius alienus*, *Camponotus spp.* and *Myrmica spp.* The plants we used to test their associative learning were *Helianthella quinquenervis* and *Ligusticum porteri*, both dominant plant species near Rocky Mountain Biological Laboratory. *H. quinquenervis* provides extrafloral nectar from which the ants can feed, and *L. porteri* is a common host for ant-tended aphids of the species *Aphis helianthi*, from which the ants acquire extrafloral nectar. Our experiment was run as follows:

Associative learning in Ant foraging

To test whether the ants were associatively learning, we created two types of baits. The first type of baits we called “Training baits” which consisted of a bamboo pole, topped with a reward for ants containing a honey solution (Fig.2a). The reward solution was 20% honey diluted in water. To create an association between scent and reward, each training bait was rubbed with the leaves of one of two plants, either *H. quinquenervis* or *L. porteri*. The leaves were randomly chosen from local plants, used until the leaf either fell apart or was lost, and the scent was standardized by swiping each leaf across the stick 20 times.

The second type of baits we called “Choice baits,” also made of bamboo, each was shaped like a Y. Each arm was topped by a honey reward (Fig. 2b). Each arm of the choice bait was scented like a different plant. One arm of the Y was scented with the *H. quinquenervis* scent, and the other with the scent of the *L. porteri*. The base of the Y was in contact with the ground was unscented, ensuring random discovery of the bait by ants foraging on the ground.

We distributed fourteen training baits along a transect, each separated by ten meters to minimize discovery of multiple baits by the same ant colony. We alternated training bait scents at each point. We attempted to control for sun exposure and elevation.

Training baits were left out for 24 hours, allowing the ants to find and acclimate to them. Following training Y shaped choice baits were added, one adjacent to each training bait (Fig. 3). A camera mounted on a brick recorded of baits by the ants. Once the choice baits were placed, we recorded the number of ants in each bait every 15-20 minutes for 1.5-2.5hours.

To test whether ant associative learning depends on plant density, we estimated the percent vegetative ground cover of both *H. quinquenervis* and *L. porteri* within a two-meter radius of each bait station. We also did a point count of ants on both plant species and recorded whether there were aphids on *Ligusticum*.

Statistical Analysis

To test whether ant discovery of training baits depended on training bait scent and the percent cover of *H. quinquenervis* and *L. porteri*, we used a generalized linear model (GLM). We included discovery as the response variable and training bait scent and *H. quinquenervis* and *L. porteri* percent cover as fixed effects. To test whether ants were more likely to discover training baits in regions of higher percent cover of *H. quinquenervis* or *L. porteri*, we also included bait scent x plant percent cover interactions in the model.

To test whether ants exhibited associative learning when foraging from the “choice baits,” we used a GLM with discovery as the response variable and choice bait scent, training bait scent, and their interaction as fixed effects.

To test whether ant recruitment to choice baits depended on the interaction between the choice bait scent and training bait scent we used a linear model with the total number of ants observed per observation as the response variable and choice bait scent, training bait scent, and their interaction as fixed effects.

All statistical analyses were conducted in R v3.3.2 (R Core Team, 2016). We constructed linear models and generalized linear models using the ‘lm()’ and ‘glm()’ functions in the ‘stats’ package, and we conducted chi-square tests using the ‘Anova()’ function in the ‘car’ package.

Results:

Effects of surrounding abundance

Ant discovery of training baits did not depend on the interaction between bait scent and the percent cover of *Helianthella* ($\chi^2=2.235$, $p = 0.135$) or *Ligusticum* ($\chi^2= 0.061$, $p = 0.804$). There were also no significant main effects of training bait scent ($\chi^2= 0.002$, $p=0.966$), or percent cover of *Ligusticum*, ($\chi^2=2.414$, $p = 0.120$) (Fig. 4), but the likelihood of discovery significantly increased with the percent cover of *Helianthella* ($\chi^2= 20.358$, $p < 0.001$) (Fig. 5).

Ant associative learning

We found a significant choice bait x training bait scent interaction ($\chi^2 = 4.573$, $p = 0.032$), where ants were more likely to discover choice baits with the same scent as the training baits. There was also a main effect of choice bait scent ($\chi^2 = 4.139$, $p = 0.042$), where ants were 13% more likely to discover choice baits that smelled like *H. quinquenervis* (vs. *L. porteri*), but the main effect of the training bait scent was not significant ($\chi^2 = 0.681$, $p = 0.409$) (Fig. 6).

Ant recruitment to baits

We found that the number of ants that recruited to the choice baits did not depend on the choice bait x training bait scent interaction ($F = 0.003$, $p = 0.960$), or the main effects of choice bait scent ($F = 0.012$, $p = 0.913$) or training bait scent ($F = 0.825$, $p = 0.366$).

Discussion:

The most compelling results of our data were that there was evidence that ants may learn associatively in the field, and that the ants preferentially foraged in areas with higher abundance of *H. quinquenervis*. Beginning with the associative learning, the data indicated that ants were more likely to discover choice baits that were the same scent as their training bait. This means that while foraging, ants may be looking for scents that they recognize as being related to resources. This agrees with the conclusions of the lab experiments previously mentioned in the introduction, and shows that this behavior persists in field conditions. From this we can imply that as resources change from area to area, the ants will change their foraging strategies to prefer sources of whatever resources there are available in their environment.

As for the effects of the higher abundance of *H. quinquenervis*, this may indicate that ants preferentially forage in areas with a higher abundance of resources. This can be extrapolated by comparing the effects of *Helianthella* abundance with the effects of *Ligusticum* abundance. In the summer of 2018 Gunnison county Colorado there were unseasonably high temperatures, and the season was drier than usual. Likely due to this, there were few if any aphids found on the wild *Ligusticum* in our transects. This means that we had recorded the abundance of plant that had resources available for ants, as well as the abundance of plant that had no or few resources for the ants. By looking at how the abundance of *Ligusticum* had no effect compared to the *Helianthella* we can guess that a difference between the plants, and the ant's response to this difference, caused a difference between their effects.

There are a couple possibilities for causes of this difference. Ants may forage more in areas that already have abundant resources. This would could mean that ants aren't only learning what plants to forage on, but also what areas to forage at. Considering that *Helianthella* had resources throughout the season, and *Ligusticum* did not, as well as the fact that the ants can learn associatively, I believe this makes the most sense. Other ant species, such as *Camponotus* have previously been shown to be capable of identifying a location by a plant scent, and modulate their behavior accordingly (Helmy & Jander 2003). This result could be evidence of similar behavior in our study species, indicating a seeking of preferred foraging grounds.

Another possibility is that the ants may prefer the scent or resources of *Helianthella* over *Ligusticum*. This is possible, and there could be reason to believe that this is true due to the nature of their relationships with ants. *Helianthella* is in a direct mutualism with ants, and could evolve to attract ants. Whereas *Ligusticum* is the parasitized host of aphids, and may want to repel ants for the detriment of those aphids. At the same time, even if they do not offer the same quantity or quality of resources to the ants, the abundance of these plants still differs across

space, which in and of itself can affect the behavior of ants. As shown by Howard (1996) in the case of some leaf cutter ants what matters more is not better resources, but rather familiar resources, leading to an assumption that density, and thus familiarity, will be more of a defining factor than quality of resources. This would indicate that preference alone may not have as strong an effect as expected. On top of this, a conclusion from this data of preference cannot be trusted due to the abnormal conditions for the *Ligusticum*, aphids, and thus resources for the ants that may have affected their foraging on these plants. To test for this preference, there would have to be more control over, and understanding of, the resources offered by each plant.

There is one more interesting implication of this finding and how it relates to our results from the associated learning portion. We saw that *Helianthella* choice baits were discovered 13% more than *Ligusticum* choice baits. It's possible that this was due to the ants being trained on that local abundance of *Helianthella*, and not being trained by resources on the *Ligusticum*. In a different year, we may have seen different results, but this also corroborates the theory that the greater discovery of baits was due to greater amounts of resources on the *Helianthella* due to how those greater resources would assist with the training of the ants.

This opens a lot of possibilities for future studies and questions. For one, it's still unclear how much of an effect local abundance had on ant learning. There could be a study of purely the density dependence, without the training bait but with the choice bait, in order to more concretely see if the ants are learning from the environment. Results could begin to clear up whether there was natural preference, or just a climactic effect on the foraging and behavior of the ants.

There could also be a study of the different VOCs in the *Helianthella* vs the *Ligusticum* to see whether the ants prefer certain scents over others. By isolating their compounds we could try to see how the scents of the two plants differ, and if they share compounds with other plants that ants like. We could also compare these with other plants that are more closely related, to see how capable of distinguishing scents the ants are.

Another study could be an observational study of ants foraging in the field, to determine in what locations there are the most ants foraging, and what the resource availability is in those locations. If ant colonies were also plotted we may find that it's not actually that ants seek out locations with more resources, but rather that it's only in the locations with more resources that the ants choose to make their colonies.

The last piece of information that would be good to know is at what organizational level the ants are learning. We found that recruitment to the baits was not related to the scent of any of the baits, meaning that the number of ants didn't change from learning, only whether a bait was discovered or not. This is interesting, because we didn't mark the ants that were on these baits, and so we couldn't know whether these were consistently the same ants, or if they were different ants. This means that it's unclear whether the ants were learning at an individual, or colony level, information that would indicate whether the ants were passing information between each other. Now that we know that the ants learn, this offers an opportunity for us to find out how much they communicate, and what they communicate.

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Literature Cited:

- Bartlett, B. R. "The Influence of Ants Upon Parasites, Predators, and Scale Insects." *Annals of the Entomological Society of America* 54, no. 4 (July 1, 1961): 543–51.
<https://doi.org/10.1093/aesa/54.4.543>.
- Bos, Nick, Fernando J. Guerrieri, and Patrizia d'Ettorre. "Significance of Chemical Recognition Cues Is Context Dependent in Ants." *Animal Behaviour* 80, no. 5 (November 2010): 839–44. <https://doi.org/10.1016/j.anbehav.2010.08.001>.
- Bronstein, Judith L. "The Contribution of Ant-Plant Protection Studies to Our Understanding of Mutualism." *Biotropica* 30, no. 2 (1998): 150–61.
- Christina D. Difonzo, David W. Ragsdale, Edward B. Radcliffe, Neil C. Gudmestad, Gary A. Secor; Seasonal Abundance of Aphid Vectors of Potato Virus Y in the Red River Valley of Minnesota and North Dakota, *Journal of Economic Entomology*, Volume 90, Issue 3, 1 June 1997, Pages 824–831, <https://doi.org/10.1093/jee/90.3.824>
- Dostál, Petr, Magdalena Březnová, Vladimíra Kozlíčková, Tomáš Herben, and Pavel Kovář. "Ant-Induced Soil Modification and Its Effect on Plant below-Ground Biomass." *Pedobiologia* 49, no. 2 (March 2005): 127–37.
<https://doi.org/10.1016/j.pedobi.2004.09.004>.
- Dupuy, Fabienne, Jean-Christophe Sandoz, Martin Giurfa, and Roxana Josens. "Individual Olfactory Learning in Camponotus Ants." *Animal Behaviour* 72, no. 5 (November 2006): 1081–91. <https://doi.org/10.1016/j.anbehav.2006.03.011>.
- Hanzawa, Frances M., Andrew J. Beattie, and David C. Culver. "Directed Dispersal: Demographic Analysis of an Ant-Seed Mutualism." *The American Naturalist* 131, no. 1 (January 1988): 1–13. <https://doi.org/10.1086/284769>.
- Helmy, O., and R. Jander. "Topochemical Learning in Black Carpenter Ants (Camponotus Pennsylvanicus)." *Insectes Sociaux* 50, no. 1 (February 1, 2003): 32–37.
<https://doi.org/10.1007/s000400300005>.

- Howard, Jerome J., Lawrence M. Henneman, Greg Cronin, Jennifer A. Fox, and Gustavo Hormiga. "Conditioning of Scouts and Recruits during Foraging by a Leaf-Cutting Ant, *Atta Colombica*." *Animal Behaviour* 52, no. 2 (August 1996): 299–306. <https://doi.org/10.1006/anbe.1996.0175>.
- Mooney, Emily H., Joseph S. Phillips, Chadwick V. Tillberg, Cheryl Sandrow, Annika S. Nelson, and Kailen A. Mooney. "Abiotic Mediation of a Mutualism Drives Herbivore Abundance." Edited by Amy Angert. *Ecology Letters* 19, no. 1 (January 2016): 37–44. <https://doi.org/10.1111/ele.12540>.
- Pavlov, I.P. (1927). *Conditional reflexes: an investigation of the physiological activity of the cerebral cortex*. Oxford, England: Oxford Univ. Press.
- Roces, F. "Odour Learning and Decision-Making during Food Collection in the Leaf-Cutting Ant, *Acromyrmex Lundii*." *Insectes Sociaux* 41, no. 3 (September 1, 1994): 235–39. <https://doi.org/10.1007/BF01242294>.
- Romero, G., & Izzo, T. (2004). Leaf damage induces ant recruitment in the Amazonian ant-plant *Hirtella myrmecophila*. *Journal of Tropical Ecology*, 20(6), 675–682. doi:10.1017/S0266467404001749
- Saverschek, N., H. Herz, M. Wagner, and F. Roces. "Avoiding Plants Unsuitable for the Symbiotic Fungus: Learning and Long-Term Memory in Leaf-Cutting Ants." *Animal Behaviour* 79, no. 3 (March 2010): 689–98. <https://doi.org/10.1016/j.anbehav.2009.12.021>.
- Styrsky, J. D., and M. D. Eubanks. "Ecological Consequences of Interactions between Ants and Honeydew-Producing Insects." *Proceedings of the Royal Society B: Biological Sciences* 274, no. 1607 (January 22, 2007): 151–64. <https://doi.org/10.1098/rspb.2006.3701>.
- Verchot, Louis V., Paulo R. Moutinho, and Eric A. Davidson. "Leaf-Cutting Ant (*Atta Sexdens*) and Nutrient Cycling: Deep Soil Inorganic Nitrogen Stocks, Mineralization, and Nitrification in Eastern Amazonia." *Soil Biology and Biochemistry* 35, no. 9 (September 2003): 1219–22. [https://doi.org/10.1016/S0038-0717\(03\)00183-4](https://doi.org/10.1016/S0038-0717(03)00183-4).

Figure Captions:

Figure 1. Location of field sites. GPS coordinates by site:

Center point Site 1 SGB Snodgrass Bend: 106 57'44.782"W 38 55'28.812"N

Center point Site 2 JFT Judd falls trail: 106 59'0.91"W 38 57'37.072"N

Center point Site 3 FTH 401 Trail head: 106 59'35.146"W 38 58'2.118"N

Center point Site 4 MML Marriage Meadow Lower: 106 59'18.187"W 38 57'53.863"N

Center point Site 5 JPLF Judd falls parking lot field: 106 59'39.956"W 38 57'58.032"N

Center point Site 6 MMU Marriage Meadow Upper: 106 59'23.219"W 38 57'59.961"N

Center point Site 7 SGL Snodgrass lower: 106 57'46.53"W 38 55'33.451"N

Center point Site 8 SGMR Snodgrass Moocow Riverside: 106 57'44.186"W 38 55'33.02"N

Figure 2a. Training bait

Figure 2b. Choice bait

Figure 3. Complete bait set

Figure 4. *Ligusticum* % ground cover by discovery

Figure 5. *Helianthella* % ground cover by discovery

Figure 6. Associative learning graph

Figures:

Figure 1:

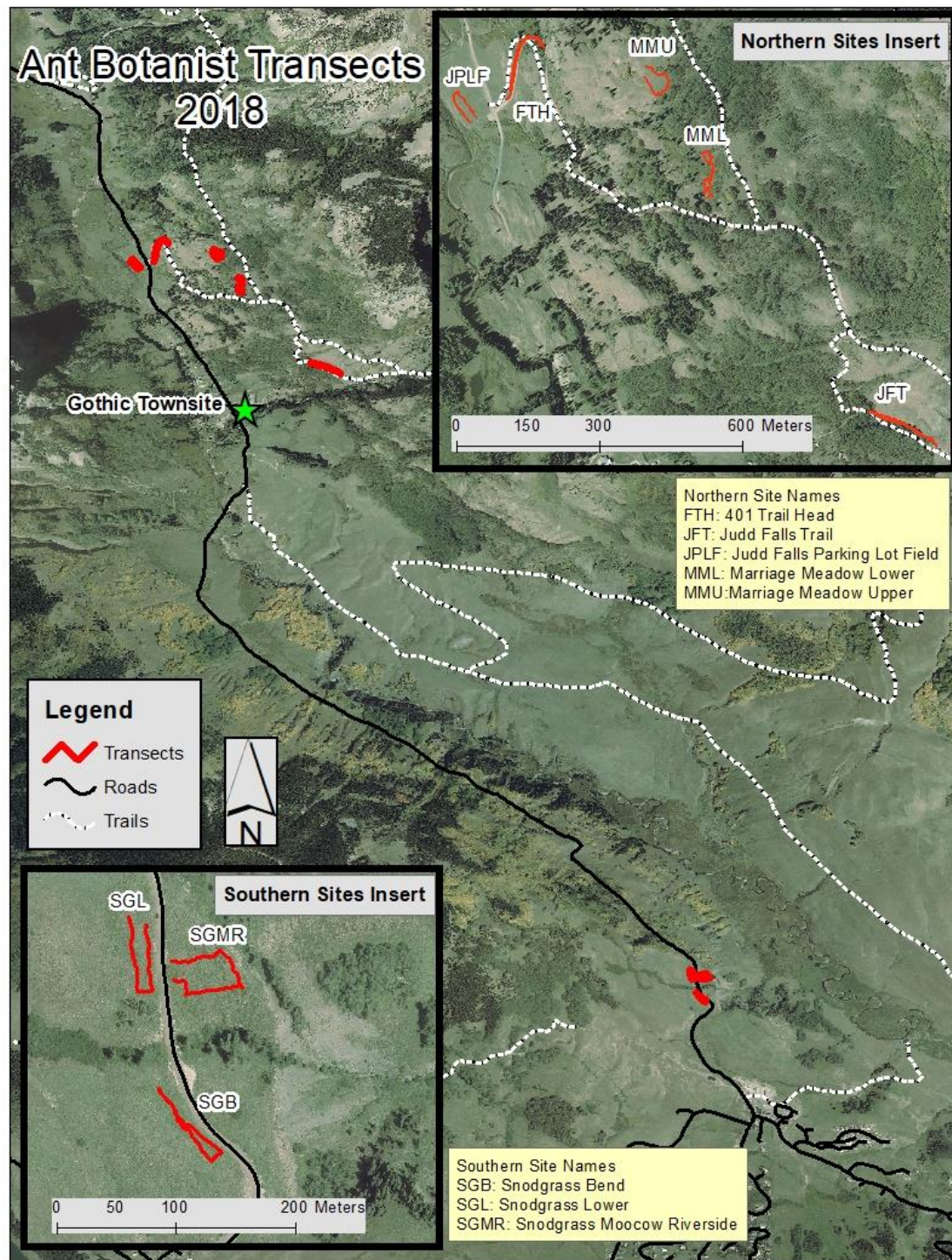


Figure 2a, 2b:

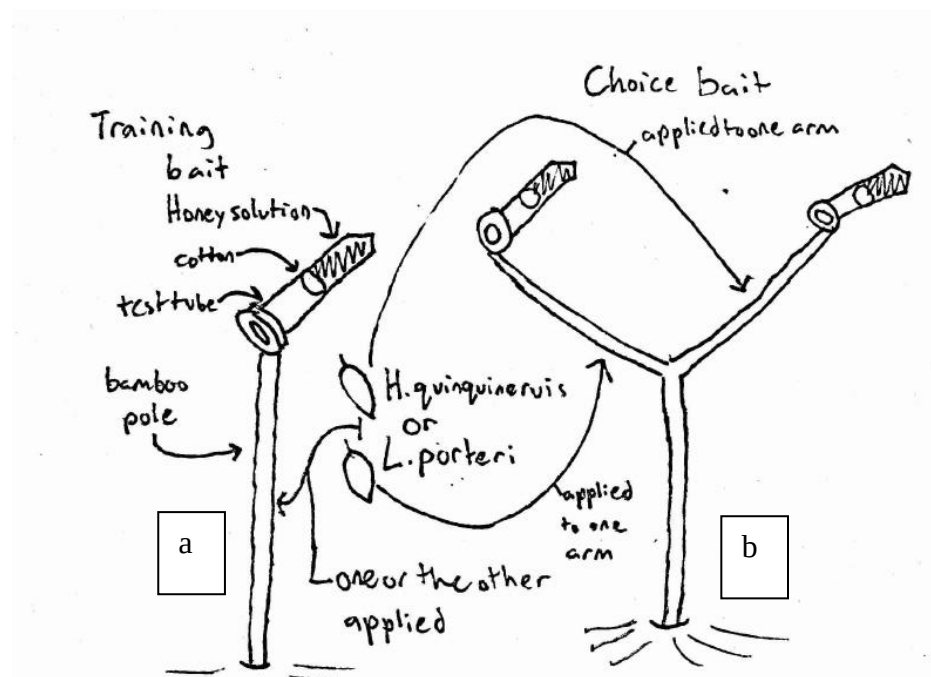


Figure 3

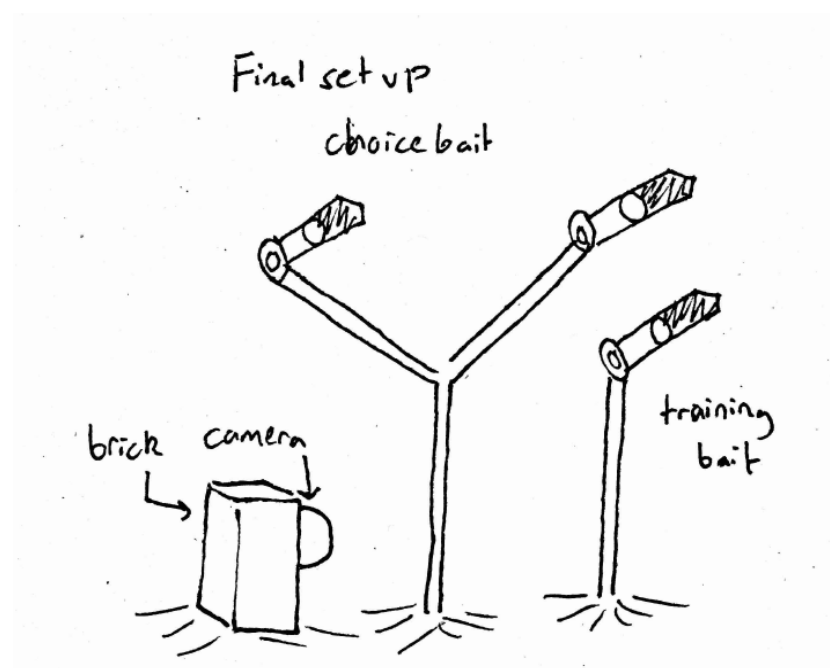


Figure 4

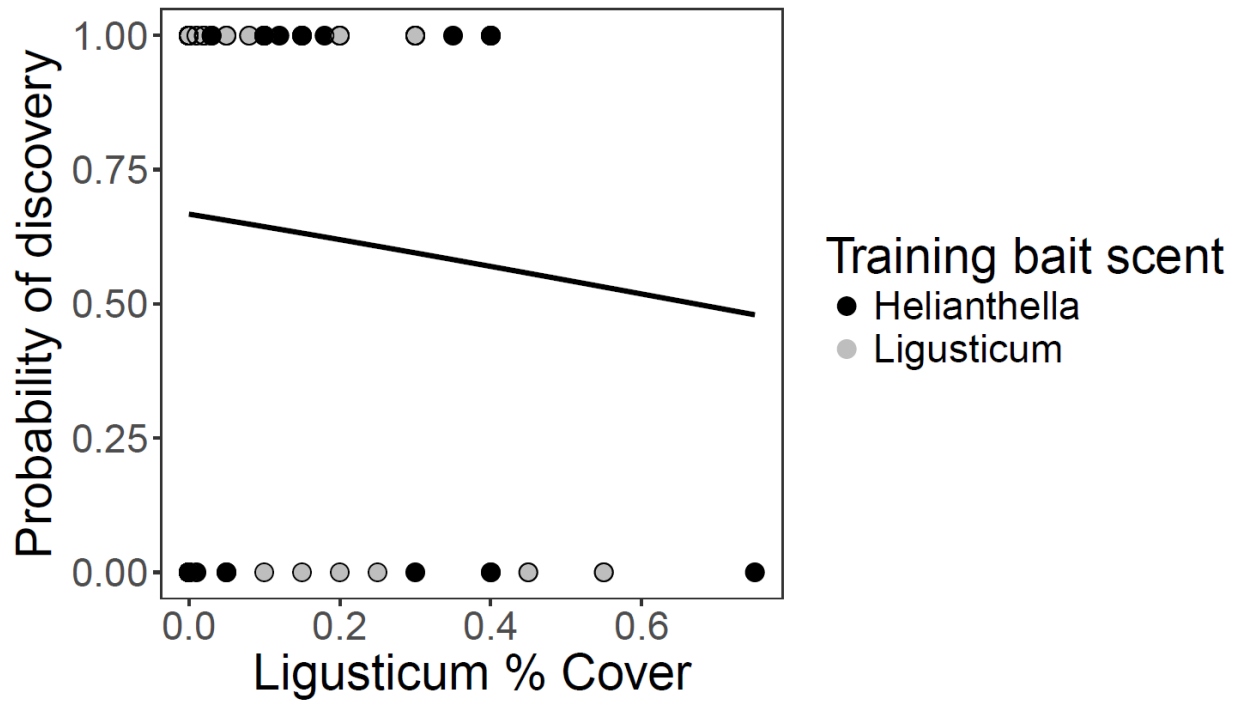


Figure 5

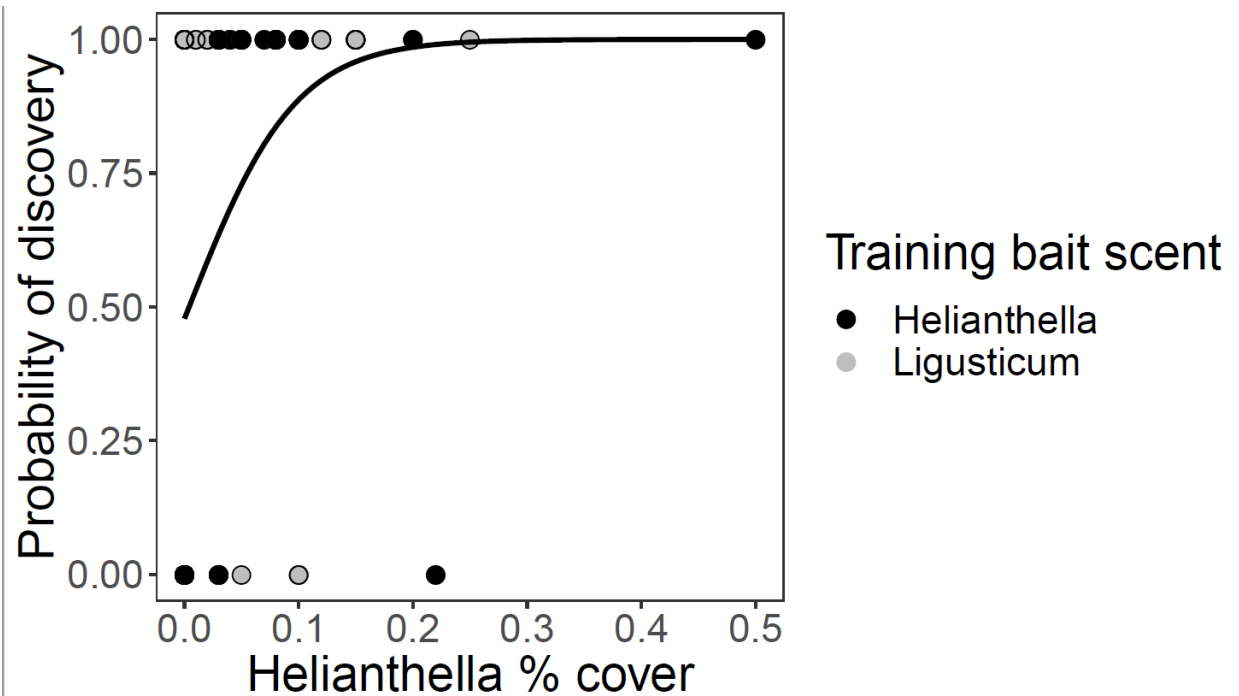


Figure 6

