

Chemical camouflage and the consequences of changing host plants in a treehopper-ant mutualism

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Abstract:

Though many prey species use camouflage to escape predation, the role of non-visual camouflage in ecological systems remains understudied, potentially overlooking important mechanisms mediating interactions between species. For instance, some insect prey species use chemical camouflage to pacify predatory ants, which can be important for mediating mutualistic relationships between insect herbivores and ants. However, the effectiveness of this anti-predation mechanism may depend on the breadth of the herbivore's diet, where herbivores that feed on many plant species can have mismatched, ineffective camouflage when migrating to new host species with different chemistry. Here I studied the potential for chemical camouflage to mediate the mutualism between phloem-feeding treehoppers and ants on different host plant species, expecting treehopper survival to decrease when transferred to new host species near ant nests. Relative to controls, treehopper survival was lower when transferred to a new host species in 3 of 4 trials. Despite lupine being a high-nitrogen host, treehopper survival was lower on lupine when transferred from both rabbitbrush and mule's ear. This was consistent with expectations from mismatched chemical camouflage. However, I did not expect that treehoppers transferred from rabbitbrush to mule's ear would have higher survival, a result that reflects other mechanisms mediating the mutualism. These results suggest that chemical camouflage may be an important factor mediating the treehopper-ant mutualism. Much additional study is needed to reveal the overall role of such non-visual types of crypsis in ecological systems.

Introduction:

Predation is often a strong selective force for prey species, which have evolved many adaptations to avoid being eaten. Camouflage is a common adaptation where prey possess traits that confer protection by hindering a predator's ability to detect and locate prey as discrete organisms (Ruxton 2009). Though visual camouflage has been well-studied, other forms of crypsis such as chemical and vibrational camouflage may be common but have yet to receive much empirical attention (Ruxton 2009). Chemical crypsis may be especially important

for herbivores avoiding ants, which are predators that detect their environment primarily through olfaction (Holldobler & Wilson, 1990). Insects such as caterpillars (Akino et al. 2004, Portugal & Trigo 2005) and treehoppers (Silveira et al. 2010) have been shown to avoid ant-predation by chemically matching the surfaces of their host plants. Some caterpillar and treehopper species also have mutualistic relationships with ants (Way 1963, Holldobler & Wilson, 1990), and chemical camouflage may be important for mediating the positive relationship between these species (Silveira et al. 2010). For instance, mutualist treehoppers that do not match the chemical composition of their host plants may become prey for ants (Silveira et al. 2010)

The effectiveness of chemical camouflage will likely depend on the generality of the herbivore's diet. Specialist herbivores feed on one plant species and only have to match the chemical background of that species. However generalist herbivores, such as treehoppers (Reithel & Billick, 2006; Reithel & Campbell, 2008), feed on many plant species and may have to adjust their cuticular chemicals to match different host species. For instance, the treehopper *Publilia modesta* benefits from mutualist ants (*Formica obscuripes*), but this depends on the host plant on which the treehoppers are born (Reithel & Billick, 2006; Reithel & Campbell, 2008). When treehoppers are born on rabbitbrush (*Chrysothamnus viscidiflorus*) they develop successfully into adults under ant protection from other predators, but treehoppers on mule ears (*Wyethia spp.*) plants must move to nearby hosts because this poor-quality plant senesces before treehoppers complete development (Reithel & Billick, 2006; Reithel & Campbell, 2008). Treehoppers on mule ears not only have to face the hazards of crawling to new host plants, but their chemical camouflage may not be effective if their new host is a species with different surface chemistry. Thus generality in host plant use may play an important role in mediating the effectiveness of chemical camouflage and the outcome of this conditional mutualism (Bronstein 1994).

In this study I evaluated the potential for chemical camouflage to mediate the mutualism between treehoppers and ants on different host plant species. I hypothesized that treehoppers that move to a new host plant species will have decreased survival because ant predation may increase for treehoppers with mismatched camouflage. I expected this effect to

vary with the degree of relatedness among plant species, as plants from different families have different chemistry (Cushman, J. H. 1991). I tested how treehopper survival is affected by migrating among three different plant species of varying relatedness: rabbitbrush, mule's ear (family Asteraceae), and lupine (family Fabaceae). Though lupine is nitrogen-fixing and may be a more nutritious host for treehoppers (Pierce 1984), treehopper survival should decrease if they have mismatched camouflage on lupine. I moved treehoppers to new host plants within and between plant species and measured treehopper survival. This study evaluates the ecological consequences of migrating to new host plants for a chemically camouflaged herbivore.

Methods:

This research was conducted in the Almont Triangle (Figure 1). The experimental meadow is dominated by *Artemisia tridentata* and *Chrysothamnus viscidiflorus* and is 100m x 200m, at an elevation of 2769m, and at latitude 38.719 and longitude -106.819 (Grinath et al. 2012). In Almont, the treehopper *Publilia modesta* is commonly tended by the ant *Formica obscuripes* (Reithel and Billick, 2006). The females oviposit on at least six genera of plants (*Wyethia spp.*, *Chrysothamnus*, *Erigeron*, *Balsamorhiza*, *Helianthella*, and *Lupinus*), between the months of May and June (Reithel and Billick, 2006). For host plants I chose *C. viscidiflorus*, which is a superior host, *Wyethia spp.*, which is an inferior senescent host and *Lupinus spp* which is a nitrogen-fixing but not so common host from a different plant family (Reithel and Billick, 2006).

In this experiment I measured treehopper survival when changing host plants. Using the three different host species, I contrasted treehopper nymph survival when transferred to a plant of the same or a different species. Pairs of similar-sized experimental plants were selected within 3 meters of ant nests to ensure ant attendance (Reithel and Billick, 2006). Plants were thoroughly inspected for the presence of treehoppers and their oviposition marks; treehoppers and stems with oviposition scars were removed at the beginning of the experiment. Source populations of treehopper nymphs were chosen at random from within the meadow. Treehopper aggregations were divided in half to make transfers; half to an alternate host species, and, as a control, half to the same host species. Nymphs were transferred using a small

paintbrush and all aggregations started with 30 nymphs. Transfers were made from *C. viscidiflorus* to *Wyethia* spp and vice versa and *C. viscidiflorus* and *Wyethia* both to *Lupinus* spp, and replicated 10 times for a total of 40 plant pairs. Transfers from *Lupinus* spp. to the other host plants were not considered because few treehoppers are born on this species at the field site. Approximately a week and a half after setting initial treehopper densities, I recorded the number of surviving nymphs on each plant.

We analyzed the abundance of surviving treehoppers on the different treatments using R 3.0.1 (R Development Core Team 2009). The data was ln-transformed to meet assumptions of normality and was analyzed using Gaussian-distributed generalized linear models (GLMs). This test is equivalent to a t-test or one way ANOVA. GLMs with other kinds of distributions were also considered, but the above test was chosen as the most appropriate type of model based on AIC scores. In the rabbitbrush to mule's ear treatment, one extreme outlying value was deleted from this test to improve model fit. Data are shown without the ln-transformation to aid interpretation of treehopper survival rates.

Additionally, I was interested in comparing treehopper survival when transferred to plants within vs. between host plant families. I compared the effects of transfers from rabbitbrush to the two alternate hosts, and from mule's ears to the two alternate hosts using Gaussian-distributed GLMs. Because different experimental plant pairs occurred throughout the meadow, the effects of local conditions on treehopper survival were accounted for by subtracting the number of surviving nymphs on control plants from the number of nymphs on treatment plants within each plant pair. This data was then z-transformed to meet assumptions of normality in the statistical analyses.

Results:

Treehopper survival was significantly affected by migrating to a new host plant species. As expected when treehopper camouflage may be mismatched, fewer treehoppers survived when transferred from rabbitbrush to lupine than when transferred between rabbitbrush (GLM: Gaussian, $t_{1,18} = 3.638$, $P = 0.002$; Figure 2a). Similarly, fewer treehoppers tended to survive transfers from mules ears to lupine than when transferred between mules ears (GLM: Gaussian, $t_{1,17} = 1.963$, $P = 0.066$; Figure 2b). Counter to expectations, more treehoppers were

found on mules ears when transferred from rabbitbrush than when transferred between rabbitbrush (GLM: Gaussian, $t_{1,17} = -2.202$, $P = 0.042$; Figure 2c). However as expected by conditions with mismatched camouflage, treehoppers were less abundant when transferred from mules ears to rabbitbrush (GLM: Gaussian, $t_{1,18} = 2.576$, $P = 0.019$; Figure 2d).

I also compared the effects of transferring treehoppers between hosts within the same plant family to hosts from different plant families, expecting that treehopper survivorship would be lower in transfers between host families. Relative to control plants, treehoppers tended to be more abundant when transferred from rabbitbrush to mules ears (within the same plant family) than to lupine (across plant families) (GLM: Gaussian, $t_{1,18} = 3.875$, $P = 0.001$; Figure 3a). However we did not find any differences in treehopper survival when transferred from mule's ear to rabbitbrush or lupine (within vs across plant families) (GLM: Gaussian, $t_{1,17} = -1.489$, $P = 0.155$; Figure 3b).

Discussion:

I found that herbivore survival was affected by migrating between species of host plants. As expected by the chemical camouflage hypothesis, survival was negatively affected when treehoppers were transferred to a new host species in 3 of the 4 trials. Relative to controls, treehopper survival was lower on lupine when they were transferred from both rabbitbrush and mule's ear. Other mutualists of ants forage on lupine because it is a nitrogen-fixing plant, allowing these herbivores to provide more nutritious rewards for ants (Hall 1991, Pierce 1984). Kay et al. (2004) suggested that membracids increase nitrogen in their honeydew to attract or appease ants. Yet treehopper survival in this experiment was lower on this high-nitrogen host, consistent with expectations from mismatched chemical camouflage.

Unexpectedly, the chemical camouflage hypothesis did not anticipate the higher treehopper survival that occurred when nymphs were transferred from rabbitbrush to mule's ear. Mule's ear was starting to senesce at the time of the experiment and was therefore expected to be the lower quality host plant (Reithel & Campbell 2008). However mule's ear is also the preferred plant for treehopper oviposition (Reithel & Campbell 2008), and may be a

higher quality plant in other traits, such as nitrogen content, which could have been beneficial for treehoppers transferred from other plant species. The specific mechanisms creating these results are uncertain and require additional study to resolve. In addition to studying plant quality, further study on treehopper camouflage is needed to confirm this as a treehopper trait in this system.

Many additional treehoppers were found on experimental plants in some treatments. These treehoppers may have migrated from nearby arrowleaf balsamroot (*Balsamorhiza sagittata*, Asteraceae) plants, which were senescing at the time of data collection, or from nearby early-senescing mule's ear. Alternatively, though I removed treehoppers from aboveground plant parts at the start of the experiment, some treehoppers may have escaped removal by sheltering within secondary ant nests along plant roots. Though these additional treehoppers may obscure survivorship results, I believe the trends found in the data reflect treehopper survivorship because I chose experimental plants that were neighbors and at random with regards to the presence of secondary ant nests, accounting for local variation in the community.

A growing number of studies are showing that chemical camouflage is an effective defense strategy (e.g. Espelie et al. 1991, Akino et al. 2004, , Portugal & Trigo 2005) and can be important in mutualistic relationships (e.g. Silveira et al. 2010). Silveira et al. (2010) demonstrated that chemical camouflage decreased ant aggression towards treehoppers, which likely helps to maintain the mutualistic relationship between ants and their potential prey. Carroll and Janzen (1973) suggested that, from the ants' perspective, homopteran insects such as treehoppers may be acting as extrafloral nectaries. Indeed, chemical camouflage could trick ants into detecting treehoppers not as insects, but as extrafloral nectaries that are part of the plant surface. Chemical analyses of the cuticles of plants and treehoppers are needed to gain a deeper understanding of the relationship between insects and their host plants (e.g. Espelie et al. 1991, Akino et al. 2004, Portugal & Trigo 2005, and Silveira et al. 2010). I expect such analyses will reveal that chemical camouflage is a widespread component of mutualistic relationships involving insects.

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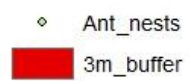
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Figure 1: Almont Triangle Map



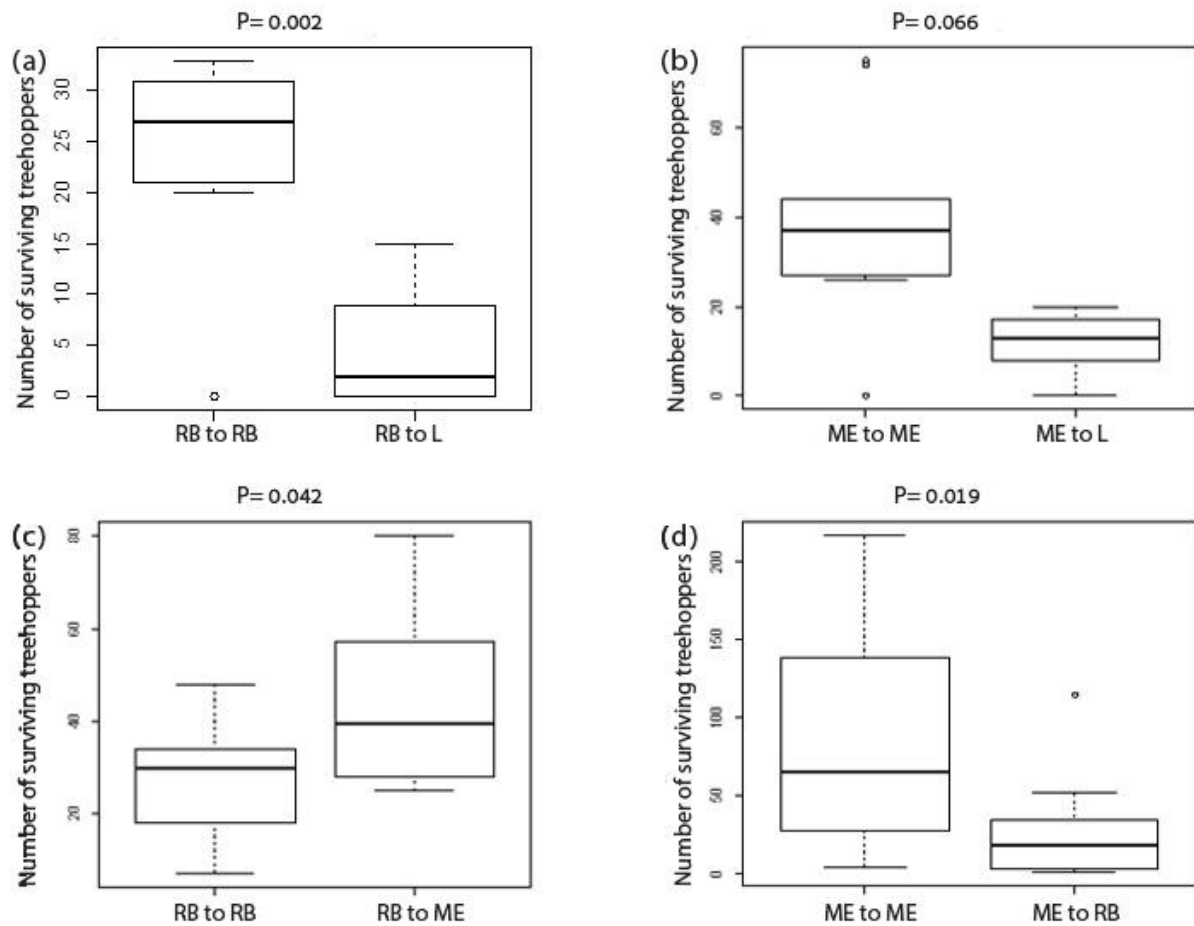


Figure 2: Survival rate results of treehopper as consequence of the transfer to different host plant species; from within Asteraceae plant family and to Fabaceae plant family. GLM: Gaussian statistical model was used to analyze the data. (a) Treehopper survival rate from rabbitbrush (RB) to lupine (L) treatment. (b) Tree hopper survival rate from mule's ear (ME) to lupine (L). (c) Tree hopper survival rate from rabbit brush to mule's ear. (d) Tree hopper survival rate from mule ear to rabbit brush.

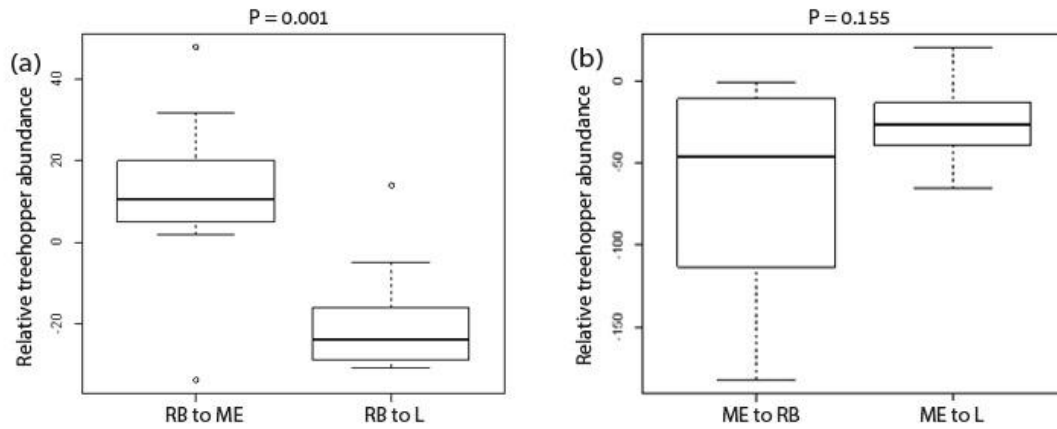


Figure 3: Comparison between the effects of transfers for each different Asteraceae family species relative to control, calculated as the number of treehoppers in treatment minus control plants. (a) Difference between the control and experimental plants for rabbitbrush (RB) to mule's ear (ME) and rabbitbrush (RB) to lupine (L) treatment. (b) Difference between the control and experimental plants for mule's ear (ME) to rabbitbrush (RB) and mule's ear (ME) to lupine (L) treatment.