

Ant-Plant Mutualisms: Mitigating Cheaters in a Cooperative System (with specific attention to Sensory Traps)

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Animals rely on a diverse set of specific signals (chemical, visual, tactile, etc.) in order to communicate and trigger behaviors, such as aggression or courtship. Ordinarily, animals respond to cues from conspecifics; however other organisms may mimic those cues to take advantage of pre-existing signaling systems. These out-of-context signals that elicit otherwise normal behavioral responses are known as sensory traps. “Sensory traps are signal mimics that exploit the adaptive neural responses of signal receivers to elicit out-of-context behaviors” (Edwards et al. 2007).

The major role of sensory traps, as argued by Edwards & Yu (2007), is to enforce cooperation between two mutualistic species, such that both parties gain equal benefit from the interaction. As with any cooperative system, cheaters, or “free-loaders”, who receive benefits disproportionate to their contribution, are a constant threat to the mutualism. With the perpetual risk of cheaters, mechanisms must evolve to enforce cooperation and deter cheating in order for mutualisms to persist.

Biologists have tried to identify mechanisms for enforcement, there is a body of literature suggesting that sensory traps could serve as a major enforcer, mostly headed by Yu and Edwards. While sensory traps attracted some initial attention, the concept itself is not empirically well supported. Although there does seem to be a fair number of examples of plants mimicking ant chemicals, as illustrated below. In addition to the sensory trapping literature, this annotated bibliography will include a more general look at studies and reviews about cheating and chemical communication in ant-plant mutualisms.

Bibliography

Bronstein, J. L., Alarcón, R., & Gerber, M. (2006). The evolution of plant-insect mutualisms. *New Phytologist*, 172, 412-428.

This review provides a general overview of plant-insect mutualisms built upon the perspective that mutualisms are simply an act of cooperation between two or more different species. The authors discuss three types of plant-insect mutualisms: pollination, protection by ants, and seed dispersal by ants. The evolution and co-evolution of each relationship is examined across several examples, distinguishing between obligate and generalist, facultative interactions. More generalized associations will tend to provide nectar rewards in exchange for protection, but usually lack the more derived traits of domatia or lipid bodies present only in more specialized associations. Bronstein et al. point out that selection for these traits is directed by the ants, but that much less is known about ant traits under selection by the host plant. This apparent asymmetry is exaggerated further in generalized ant-plant associations, giving ants the upper hand. With this in mind, Bronstein et al. delves into a more theoretical discussion about cheaters, such as ants that steal nectar without protecting the plant, and conditions under which mutualisms are maintained. In addition, they clearly distinguish between

“cheating from within” and “from without,” and how each type of cheating can influence the stability of a mutualism (p. 422). Curiously, there is little evidence for the existence of traits that prevent cheating, much less than would be expected from theory. They conclude their review by calling upon the development of more evolutionary theory and models to address this and other unresolved questions, and to synthesize the rapid influx of ant-insect knowledge.

Brouat, C., McKey, D., Bessièrè, J.-M., Pascal, L., & Hossaert-McKey, M. (2000). Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: Preliminary results on *Leonardoxa* (Fabaceae: Caesalpinioideae) and *Petalomyrmex* (Formicidae: Formicinae). *Acta Oecologica*, 21 (6), 349–357.

The aim of this study was to determine whether *Petalomyrmex* ants require a chemical cue to discriminate the young leaves of *Leonardoxa*, which they stereotypically patrol and are exclusively and obligately associated with. The authors examine this system from both a proximate and ultimate perspective, asking first if a chemical is the mechanism by which ants “defend” and more ultimately, whether such a mechanism originated solely to attract ants. To answer these questions, the authors tested ant visitation times to leaf extracts of mature and young leaves, and also to extracts from young and mature leaves of a non-obligate ant tended plant in the same genus (more basal). They then determined the chemical composition in each leaf type in order to identify compounds correlated with high ant visitation rates. Ants consistently spent more time at the younger leaf extract, consistent with their predictions. Also, a single compound, methyl salicylate, was found to be more abundant in the young leaves relative to the mature leaves, and was also 100 times higher in the obligate plant than in the facultative ant-plant. While they explain that methyl salicylate has been shown to have many different functions in plants, the exceptionally high concentrations in the young leaves of this obligate ant-plant suggest that methyl salicylate acquired a new function in the evolution of this specialized ant-plant mutualism. They go on to suggest that methyl salicylate acts only as a cue, and not a resource, with the direct reward for the ant being increased host growth and survival. In contrast to the sensory trap literature, this system represents an example in which ant and plant interests are aligned. The experiments outlined in this study are very well done and should stand as a model to testing chemical signaling in ant-plant systems.

Duffield, R. M., & Blum, M. S. (1974). Methyl 6-methyl salicylate: identification and function in a ponerine ant (*Gnamptogenys pleurodon*). *Experientia*, 31 (4), 466.

This is the classic paper cited by most of the ant-plant chemical signaling papers, and is especially noted in the studies of Edwards (2007) and Brouat (2000) as a target of ant-mimicking plant compounds. Duffield and Blum identified methyl 6-methyl salicylate as an alarm pheromone in a primitive ant, *Gnamptogenys pleurodon*. In this study, they extract the primary compound found in adult worker heads and observed that conspecifics become highly aggressive when exposed to that compound. They also demonstrated that this compound is held primarily in the mandibular gland of worker heads. They note that this same compound has been identified in *Camponotus* (Formicidae) males and is involved in swarming during nuptial flights. They use this set of information to illustrate that methyl 6-methyl salicylate has high potential to be used in diverse behaviors and forms of communication across ant

species. This hypothesis is supported by more recent papers suggesting its involvement in plant defense in ant-plant associations.

Edwards, D. P., & Yu, D. W. (2007). The roles of sensory traps in the origin, maintenance, and breakdown of mutualism. *Behavioral Ecology and Sociobiology* , 61, 1321–1327.

This review lays the theoretical framework for subsequent studies on sensory traps in ant-plant interactions. Edwards and Yu discuss how “cheaters” have the potential to invade mutualisms, breaking down an ESS. They go on to explain the phenomenon of “host sanctions”, which are tactics employed by the host that coerce the “cheater” into cooperating by threatening to terminate the interaction. They devote the rest of the review to elaborating the various ways in which sensory traps, i.e. chemical mimics, could be utilized in animal-plant mutualisms, specifically discussing: (1) assembling host and mutualist together in space; (2) smoothing over temporal lapses in reciprocating benefits; (3) enforcing defectors into cooperating; and (4) reducing the costs to one partner. This paper is a good place to start when exploring Edward’s body of work, and also for gaining an understanding of how sensory traps might function from an evolutionary standpoint. In addition, this review sits in contrast to the honest-signaling theory presented by Zahavi (1975), but emphasizes that the degree of signaling honesty or trickery may depend on the nature of the interaction, as in pollinator-plant interactions versus ant-plant interactions.

Edwards, D. P., Arauco, R., Hassall, M., Sutherland, W. J., Chamberlain, K., Wadhams, L. J., et al. (2007). Protection in an ant-plant mutualism: an adaptation or a sensory trap? *Animal Behavior* , 74, 377-385.

This article lays the foundation for work on sensory traps in ant-plant systems. Edwards et al. examines the ant-plant association of *Cordia nodosa* and *Allomerus octoarticulatus*, a mutualism in which ants patrol young leaves and the plant provides domatia and food bodies. The authors hypothesize that young leaves produce chemicals that mimic brood, stimulating protective, nursing behaviors out of context. Their hypothesis rests on the assumption that there is no caste system, meaning patrollers and nurses should be equally attracted to the odor of young leaves and brood. To test their hypothesis, they perform behavioral assays, allowing ants to choose between volatiles from (1) young leaves vs. ant brood, and (2) young leaves vs. mature leaves. Their results had poor support for the sensory trap hypothesis, and suggested the existence of behavioral castes. A major problem in their results is that little difference between ants’ preference for young versus mature leaves was found, suggesting that this method may not be an effective test. Furthermore, they use a Y-tube for bioassays, which I assume was cleaned after each trial, however there is no mention of such precautions to eliminate scent trails that might have skewed results. In addition to behavioral assays, Edwards et al. ran gas chromatography (GC) on young and mature leaves and brood to identify chemicals common to young leaves and brood. Although the results from GC suggested some shared compounds between young leaves and brood, their results point to another form of sensory trap other than brood mimicking compounds. The high quantity of methyl salicylate in young leaves, released by the plant usually after herbivory, lead Edwards et al. to suggest that this chemical acts as a constant alert to patrollers. This form of “crying wolf” serves as a sensory trap to ants, eliciting patrolling despite the absence of herbivory. This paper is strikingly

similar to the Brouat 2000 paper, but frames the question in terms of sensory trap theory. This study should be repeated using more sensitive testing methods, and perhaps using a single-species ant-plant association.

Edwards, D. P., Hassall, M., Sutherland, W. J., & Yu, D. W. (2006). Assembling a mutualism: ant symbionts locate their host plants by detecting volatile chemicals. *Insectes Sociaux*, 53, 172-176.

This study addresses the way in which plants and their host ants are tied together using chemical signals. Edwards et al. focus on how independent dispersal of host plant seeds and ant queens leads to transmission of the mutualism to the next generation. They use a myrmecophyte (plant) *Cordia nodosa* as their model system. *C. nodosa* hosts only one ant colony per plant, but appears to host a variety of different ant species in nature, including *Allomerus octoarticulatus* and four species of *Azteca*. Edwards et al. perform a simple behavioral bioassay in which the queens of each species are forced to decide between the volatiles of *C. nodosa* and the volatiles of a non-host plant. Their results show that queens of all species significantly preferred chemicals from their host plant. While their study illustrates that queens of mutualistic ants prefer their host plant, they do not show that non-mutualistic ants lack the ability to distinguish or show a preference. It may be that all ants are attracted to the odor emitted by *C. nodosa* saplings. Additionally, generalizations made about ant-plant associations with multiple ant species are more difficult to interpret than in specialized ant-plant systems, especially when the goal is to identify specific chemical targets. A follow-up study might look at each ant species' physical response to specific plant volatiles and determine if there are any compounds that consistently elicit a response across ant species. This paper is a good starting point in testing theories and scenarios of how ant-plant mutualisms could have evolved, but does not address alternatives, such as visual cues, to explain how ants locate and choose plants in nature. Questions pertaining to sensory trapping or cheating are not directly relevant in this paper, as coordination of chemical signals represents a cooperative interaction.

Edwards, D. P., Hassall, M., Sutherland, W. J., & Yu, D. W. (2006). Selection for protection in an ant-plant mutualism: host sanctions, host modularity, and the principal-agent game. *Proceedings of the Royal Society B*, 273, 595-602.

This study supplements Foster et al.'s (2006) paper concerning partner choice and host sanctions, however the empirical data in this study does not support their theoretical arguments. Using the ant-plant system *Cordia nodosa* and *Allomerus octoarticulatus*, the authors caused direct damage to the two youngest leaves with no fitness cost to the plant. They argue that the spatial arrangement of domatia, rather than increased growth of domatia, are of primary concern to ant fitness, and a reason for not detecting an effect. The ideas presented in this paper are very relevant and could be viable explanations for how mutualisms are maintained, however this is not the best source of empirical tests proving such.

Ferrière, R., Bronstein, J. L., Rinaldi, S., Law, R., & Gauduchon, M. (2002). Cheating and the evolutionary stability of mutualisms. *Proceedings of the Royal Society of London B*, 269, 773-780.

This paper uses a mathematical modeling approach to predict the evolutionary trajectory of mutualistic relationships when threatened by cheaters. The authors create a model to describe obligate mutualistic interactions in asexual organisms. They use "commodities" as the reward or resource provided by one

player (such as extra-floral nectaries on plants), and track the competition over those resources between different phenotypes. Individuals who are more effective at extracting commodities could spread to fixation, and lead to extinction of the mutualism; however, when mutant cheater phenotypes first appear, a frequency dependent equilibrium of co-existence can be reached as long as each player remain in the “region of persistence,” as dictated by the model. Under conditions of weak competitive asymmetry, the host reduces the commodity to compensate for the cheaters, but the non-cheater phenotype is selected to become a better mutualist. Ironically, this means that the occurrence of cheaters can lead to an overall benefit for the non-cheater by increasing efficacy of the mutualist-host relationship. The evolutionary trajectories for mutualisms will vary depending on the costs of investing in commodities and the degree of competitive asymmetry between mutant cheaters. This model can be loosely applied to ant-plant associations, however a cheater would have to be identified in nature in order to test empirically. Nonetheless, this paper provides a theoretical basis for the evolution of obligate ant-plant mutualisms, and leaves some space for the sensory trap hypothesis, as a mutant phenotype could use chemical traps to cheat, although there is no explicit mention of sensory trapping.

Foster, K. R., & Kokko, H. (2006). Cheating can stabilize cooperation in mutualisms. *Proceedings of the Royal Society B*, 273, 2233-2239.

While many of the papers in this bibliography attempt to explain the proximate mechanisms of enforcing cooperation in ant-plant mutualisms, this paper provides a theoretical counter point. Foster et al.'s model argues that cheaters are required in order to maintain a stable mutualism. By maintaining variability in the symbiont gene pool, and therefore the ongoing production of cheaters and non-cheaters, this allows the plant to select for and reinforce cooperative phenotypes. Without this variability, as in with lekking and mate choice, there can be no selection to maintain a particular trait, such as plumage color or herbivore defense. Plants' mechanisms for selection, or “choosing” their symbiont, might be achieved via the mechanisms elaborated on in this collection of literature, although no one has looked specifically at partner choice ant-plant systems. Previous studies on legumes and rhizobia have paved the way in host sanctioning and partner choice work, and are used as a prototype in this paper.

Gammans, N., Bullock, J. M., Gibbons, H., & Schönrogge, K. (2006). Reaction of mutualistic and granivorous ants to *Ulex* eliasome chemicals. *Journal of Chemical Ecology*, 32, 1935-1947.

In this study, Gammans et al. ask whether granivorous ants and seed dispersing ants respond to eliasome chemicals equally. A non-essential piece of the seed, eliasomes are food bodies high in lipids and proteins that a seed dispersing ant consumes before discarding seed. They hypothesize that eliasome chemicals will attract seed dispersing ants, but not granivorous ants, who consume the entire seed. Using behavioral assays of eliasome chemical fractions, they found results consistent with their hypothesis. In addition, they found that seed-dispersing ants were especially sensitive to diglycerides, and depended on that chemical cue to locate seeds with eliasomes, as visual cues were not sufficient. Gammans et al.'s experiments demonstrate a type of cooperation between species, and suggests a process of co-evolution between the two study species. This is a well thought out study looking at proximate mechanisms of seed dispersal in ants, but does not address themes of cheating. Their

methods are exhaustive of alternative hypotheses and make a fair comparison with a granivorous ant species. An interesting direction for the study of seed-dispersing ant mutualisms would be to ask whether some granivorous ants could evolve heightened sensitivity to diglycerides or whether seed-dispersing ants cheat by not transporting seeds. Given the literature on cheating in mutualisms, one might expect these phenomena to exist in nature.

Ghazoul, J. (2001). Can floral repellents pre-empt potential ant-plant conflicts? *Ecology Letters*, 4, 295-299.

Ghazoul's study on floral ant-repellents begins to explain how plants have adapted to mediate conflict with their associated ants. With flowers as a rich nectar source, why don't nectar-tending ants visit flowers? What mechanism prevents ants from taking floral nectar? If ants were not deterred from flowers, they would act as parasites on the plant-pollinator mutualism and deter pollinator visitation. Therefore, we expect to find floral ant-repellants on plants extra-floral nectaries. To determine whether such repellants exist, Ghazoul wiped un-opened flowers from a myrmecophyte *Acacia* with mature flowers and found reduced visitation from controls. In addition, Ghazoul also wiped mature flowers onto half of a petri dish and left the other side untreated. He also created several other plant treatments to control for unfamiliar odors. Again, ants preferred the control side, and even exhibited aggression on the flower treated side. Interestingly, ants were even aggressive towards floral chemicals of non-myrmecophyte species, suggesting that evolution of ant repellents occurred early on in a common angiosperm ancestor. Why haven't ants developed resistance to such repellants? Ghazoul believes that those repellants must mimic ant alarm pheromones, which would explain the aggression observed in the experiments. More work needs to be done to identify which chemicals might serve as these alarm mimics, and further describe ant reactions to these stimuli. This is an excellent study for understanding the proximate mechanisms by which plants mediate cheating behaviors, such as nectar stealing or flower castration.

Inui, Y., & Itioka, T. (2007). Species-specific leaf volatile compounds of obligate *Macaranga* myrmecophytes and host-specific aggressiveness of symbiotic *crematogaster* ants. *Journal of Chemical Ecology*, 33, 2054-2063.

Inui and Itioka perform a simple experiment on the *Macaranga*-*Crematogaster* system. At their site, four different *Macaranga* plant species, each with their own specific ant symbiont, are found in close proximity. The ant symbiont defends its host using aggressive tactics of recruitment followed by biting, however there seems to be some variation in level and type of aggression between different species. Aggression is stimulated by leaf damage, but ants also respond to damage of neighboring plants of a different species. This observation suggests that defense signals are released as volatiles, however one would expect that ants should not respond equally to the volatiles of neighboring plants. To test whether there has been co-speciation, or evolution of specialized communication, Inui and Itioka introduced fresh non-host leaf fragments and host leaf fragments and measured aggression. They found that ants were most aggressive in response to their host leaf. They also ran a mass-spec and GC to identify species specific compounds in the four *Macaranga* species. Although they conclude that their study demonstrates species specific responses adapted to host herbivores, I find their conclusions

problematic or incomplete. A heightened response to host plant volatiles may be a question of familiarity rather than evolved sensitivity. Their sample sizes were also fairly low, n=10 per species, and could have been more convincing with more trials. In addition, an explanation for their observation that ants respond to neighboring plants, but less strongly, may be the artifact of a more diluted signal. As with other studies, a more fine-tuned experiment directly linking specific chemicals to specific behaviors would be more informative. Despite these issues, I think this is a well done study and fits into the empirical evidence showing cooperation between two species on an evolutionary time scale.

Izzo, T. J., & Vasconcelos, H. L. (2002). Cheating the cheater: domatia loss minimizes the effects of ant castration in an Amazonian ant-plant. *Oecologia*, 2002, 200-205.

In this obligate ant-plant system, ants nest in domatia at the base of young leaves and defend those leaves against herbivory. Ants destroy flower buds on the young shoots they patrol, thereby castrating the plant to increase resources devoted to domatia growth. However, the plant aborts the domatia of older leaves, causing ants to abandon these mature branches. Without the presence of ants, the plant produces flowers successfully on these older branches, a strategy the authors term “cheating the cheater.” In their series of experiments, they show that young leaves rely on ants for herbivore defense by ant removal treatments. They also show that the plant suffers reproductive loss when ants are present. The plant pays a direct fitness loss (reproductive output) in exchange for indirect increase in fitness from herbivore defense. They also point out that domatia abortion is not common across the genus under study (*Hirtella*), suggesting that this mechanism originated in this particular ant plant as a way to mitigate the ant’s castration strategy. Overall, this study provides a good example of how one could approach the question of cheating by combining, phylogenetic, behavioral and ecological (herbivory) components to build a thorough understanding of tensions in mutualisms.

Kautz, S., Lumbsch, H. T., Ward, P. S., & Heil, M. (2009). How to prevent cheating: a digestive specialization ties mutualistic plant-ants to their ant-plant partners. *Evolution*, 63 (4), 839–853.

Comparable to the study done by Izzo et al. (2002), this study attempts to answer the question of how to avoid cheaters with a multi-faceted approach. The authors aim to characterize a mechanism that stabilizes the mutualism between *Acacia* trees and *Pseudomyrmex* ants. Certain obligate *Acacia* plants have enzymatic activity that cleaves sucrose into fructose and glucose, which is ultimately released at extra-floral nectaries. By producing the by-products of sucrose, *Acacia* trees are able to specialize their reward and stay under the radar of generalists in search of sucrose, and thereby reinforce cooperation between the two mutualists. Obligate mutualist ants have lost the ability to produce this sucrose-cleaving enzyme as the plant gained that ability. In speculation of whether this enzymatic specialization is a response to cheating, the authors (1) determine the feeding preference of mutualists, exploiters, and generalist ants; (2) determine the amount of enzyme activity in each guild; and (3) determine the phylogeny of *Pseudomyrmex* in reference to results from the first two aims. Ultimately, they would like to see if exploiters are derived from mutualists or from generalists. They find that the majority of cheaters are derived from generalists, and are therefore classified as “parasites.” Apart from providing an in depth background section, this study merges several fields of study to address a more theoretically

based question about cheating in mutualisms. The experiments are well thought out and address exceptions to their results without deflating their conclusions.

Youngsteadt, E., Nojima, S., Häberlein, C., Schulz, S., & Schal, C. (2008). Seed odor mediates an obligate ant-plant mutualism in Amazonian rainforests. *Proceedings of the National Academy of Sciences*, 105 (12), 4571-4575.

This study is one of the few empirical tests examining ant-chemical mimicry in a host plant. The methods in this study clearly test for correlations between seed carrying behaviors and seed chemical composition. Using both behavioral assays and qualitative analysis of seed chemical composition, they identify a potential ant-attractant chemical, illustrating that some plants may be utilizing ant-like volatiles to increase seed dispersion. Despite their conclusions, their work does not address the sensory trap hypothesis directly, but rather provides a candidate system for the study of sensory traps. More work needs to be done to determine whether this ant-plant relationship is in fact a mutualism, and what benefits might be gained by the ants from collecting certain seeds.