

## NEWS AND VIEWS

## PERSPECTIVE

**Proteases hold the key to an exclusive mutualism**

JUDITH L. BRONSTEIN

*Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, 85745, USA*

**Mutualisms, cooperative interactions between species, generally involve an economic exchange: species exchange commodities that are cheap for them to provide, for ones that cannot be obtained affordably or at all. But these associations can only succeed if effective partners can be enticed to interact. In some mutualisms, partners can actively seek one another out. However, plants, which use mutualists for a wide array of essential life history functions, do not have this option. Instead, natural selection has repeatedly favoured the evolution of rewards – nutritional substances (such as sugar-rich nectar and fleshy fruit) with which plants attract certain organisms whose feeding activities can then be co-opted for their own benefit. The trouble with rewards, however, is that they are usually also attractive to organisms that confer no benefits at all. Losing rewards to ‘exploiters’ makes a plant immediately less attractive to the mutualists it requires; if the reward cannot be renewed quickly (or at all), then mutualistic service is precluded entirely. Thus, it is in plants’ interests to either restrict rewards to only the most beneficial partners or somehow punish or deter exploiters. Yet, at least in cases where the rewards are highly nutritious, we can expect counter-selection for exploiter traits that permit them to skirt such control. How, then, can mutualisms persist? In this issue, Orona-Tamayo *et al.* (2013) describe a remarkable adaptation that safeguards one particularly costly reward from nonmutualists. Their study helps to explain the evolutionary success of an iconic interaction and illuminates one way in which mutualism as a whole can persist in the face of exploitation.**

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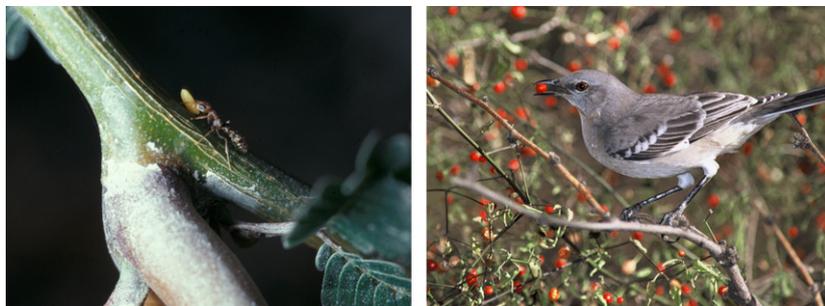
The interaction between certain Central American acacia species and the ants that protect them from consumers (Fig. 1) was first described by Thomas Belt, a geologist and explorer, in his 1874 book, *The Naturalist in Nicaragua*. The

ants (members of the *Pseudomyrmex ferrugineus* group) nest within swollen thorns and fiercely attack animals that touch or stray onto their home plant. Belt recorded the presence of unusual ‘food bodies’ (sometimes termed Beltian bodies) produced at the tips of certain leaves, postulating that they had evolved for ants to feed their larvae. Belt’s argument was dismissed by prominent ant biologists for almost a century; William Morton Wheeler is said to have scoffed that ‘saying ants benefits plants is like saying fleas benefit dogs’. However, classic experiments by Janzen (1966, 1967) and others ultimately vindicated Belt’s interpretations. Food bodies in fact make up a significant portion of the resident ants’ diet. Their nutritional value is undeniable: they are rich in lipids and proteins and contain several essential amino acids as well (Heil *et al.* 2004). If they are so valuable, however, then why are not they regularly stolen by other animals? Conventional wisdom has generally held that the mutualistic ants’ ferocity prevents other arthropods from feeding on their plants. However, more recent work has revealed a handful of other, nonmutualistic inhabitants that do in fact successfully elude the defenders.

Martin Heil and his collaborators have been exploring this conundrum in depth in two Mexican swollen-thorn acacias, *Acacia hindsii* and *A. cornigera*. Having recently shown that these species protect extrafloral nectar, another food resource produced for ants, by chemical means (summarized by Heil *et al.* 2010), Orona-Tamayo *et al.* (2013) started from the assumption that food bodies too would exhibit some form of chemical protection. Integrating evidence from proteomics, biochemistry, and both plant and ant physiology, the authors here elegantly demonstrate that acacia food bodies exhibit features that prevent all but the larvae of the obligate ant mutualists from digesting them. They first documented the presence of specific, biologically active protease inhibitors within the food bodies. They then tested whether three potential consumers – the obligate mutualists, a congeneric ant exploiter that lives on but does not defend these plants and nonadapted seed-feeding beetles – are able to digest food bodies. Larval ant mutualists exhibited a high level of proteolytic activity that rendered them insensitive to the food bodies’ protease inhibitors; the exploiter ant exhibited proteolytic activity reduced enough to make food body consumption possible but not particularly efficient. Digestion by nonadapted beetles, in comparison, was strongly inhibited by food body protease inhibitors. Thus, Orona-Tamayo *et al.* (2013) conclude that these acacias are able to reward mutualists with valuable food, while simultaneously making it impossible for nonadapted exploiters to digest.

The work of Orona-Tamayo *et al.* (2013) lends some credence to a common view of mutualisms: that they can

Correspondence: Judith L. Bronstein, Fax: 520-621-9190; E-mail: judieb@email.arizona.edu



**Fig. 1** Mutualistic ant (*Pseudomyrmex ferrugineus*) carrying an *Acacia collinsii* food body and mutualistic mocking bird (*Mimus polyglottos*) feeding on a chiltepin chilli (*Capsicum annuum* var. *glabriusculum*). Ant/Acacia photograph provided by Martin Heil and bird/chilli photograph provided by Joshua Tewksbury.

only persist if traits exist that protect them from disruption from exploiters. However, it has proven surprisingly difficult to find examples in which exploiters are deterred as effectively as shown here. Indeed, it is far easier to identify mutualisms that persist even while apparently overrun with exploiters. Interestingly, the most similar case to the one documented here comes from a dramatically different mutualism. Tewksbury & Nabhan (2001) have shown that capsaicin, the compound that gives chilli fruits their fierce heat, has no effect on chillies' mutualistic seed-dispersing birds (Fig. 1): they apparently lack the neural receptors necessary to detect this chemical. In contrast, small mammals – which destroy chilli seeds when they consume the fruits – find capsaicin to be a potent deterrent.

While differing in details, this pair of case studies of chemically mediated filters raises two critical points about how we study mutualism. First, molecular, chemical and physiological tools, particularly in combination with behavioural studies, hold tremendous potential to reveal the mechanistic underpinnings of these interactions. The surprising rarity of such studies may be the most straightforward explanation for why fine-tuned exploiter control seems to be rare in nature. Second, these studies tell us that to understand how mutualism persists in the face of exploitation, it is essential to move beyond the standard pairwise perspective. Indeed, as the mutualists within the systems studied both by Tewksbury & Nabhan (2001) and by Orona-Tamayo *et al.* (2013) were entirely unable to detect the substances that deterred antagonists, exploiter control would have entirely eluded detection if a multispecies perspective had not been adopted.

While we should recognize that their very effectiveness may render certain exploiter exclusion mechanisms invisible to us, the apparently fine-tuned exploiter control documented by Orona-Tamayo *et al.* (2013) is, nevertheless, unlikely to be widespread in nature. It is reasonable to hypothesize that they are most likely to be found in mutualisms that show the unusual level of specialization exhibited by the acacia-ant system, that is, in which there is an obligate, specialized mutualist, no more than a small handful of exploiters, and relatively high investment in rewards. As an informative comparison, consider more generalized ant defensive mutualisms. Plants in over one hundred families secrete extrafloral nectar, which rewards diverse species of aggressive ants; these ants do not nest on the plants and feed upon both animal prey and extrafloral nectar

from many plant species (Marazzi *et al.* 2013). Although detailed chemical studies have yet to be conducted, there is minimal evidence that extrafloral nectar in these generalized, facultative mutualisms is restricted either physically or chemically to the subset of consumers that are effective defenders. Rather, it appears that (i) the costs to plants of producing this reward are quite low and (ii) the fitness costs inflicted by losing some of it to nonmutualistic consumers are quite low as well. Indeed, the surprisingly low cost of being cheated may be the simplest, most general explanation for how mutualisms can persist in an apparently hostile world (Bronstein 2001).

One of the strongest and most surprising conclusions that Orona-Tamayo *et al.* (2013) draw regards the role of co-evolution in shaping the traits they have so elegantly documented. The mutualism between swollen-thorn acacias and aggressive, specialized ants has long been treated as a classic case of co-evolution. This interpretation has been argued on the basis of features such as its symmetrical specificity and the apparently close match of plant and ant traits. The intricate physiological and biochemical traits documented here would certainly lead one to assume that they too are the product of co-evolution. Yet, the authors carefully build a convincing argument that this is not in fact the case. They provide evidence that this is more likely an interaction between pre-adapted partners. Co-evolutionary processes operating on ant proteases and plant protease inhibitors may have reinforced this mutualism, they argue, but are not the best explanation for the rewards' exclusivity.

After many years of studying ant acacias and watching his careful approach to co-evolutionary analysis broadened to the point of hand-waving arguments, Daniel Janzen reacted with a short, acerbic, now-classic article challenging us to hold co-evolutionary hypotheses to the highest possible scientific standards (Janzen 1980). In this new study of the same system, Orona-Tamayo *et al.* (2013) have met that challenge admirably.

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- The first author contributed exclusively to this perspective.
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