

Ants as Mutualists

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6.1 Introduction

The historical emphasis on the ecological and evolutionary importance of antagonistic interactions such as competition, predation, and parasitism is increasingly informed by a recognition of facilitative and mutualistic interactions where one or both participants receive a net benefit (Bertness and Callaway 1994; Bruno *et al.* 2003; Grosholz 2005; Stachowicz 2001). Interactions between ants and their partners provide some of the best examples of the reciprocally beneficial interactions (Bronstein 1998) and, in particular, the mutualisms that play critical roles in structuring community composition and functioning (e.g. Christian 2001; Kaplan and Eubanks 2005; Mooney 2007; O'Dowd *et al.* 2003; Poulsen and Currie 2006; Wimp and Whitham 2001). Interactions between ants and their partners date to 45–60 Mya (Poulsen and Currie 2006; Stadler and Dixon 2005) and are critical to understanding the evolution and ecological success of ants as a taxon. The rewards provided by mutualists can increase the survival and reproduction of ants and colonies, provide the fuel that allows ants to collect new resources and engage in aggressive behaviours (Davidson 1998), and encourage colonies to reallocate resources towards particular responsibilities and/or locations. Here, we describe the currencies and dynamics of these mutualistic interactions, and highlight recent developments in our understanding of ants' participation in mutualisms.

The complexity and breadth of this topic warrant two caveats. First, the dynamics of particular ant mutualisms have been the focus of substantive reviews (e.g. refer to plant protection in Bronstein 1998, Heil and McKey 2003; insect tending in Pierce

et al. 2002, Stadler and Dixon 2005, Way 1963; seed dispersal in Giladi 2006; ant–plant symbioses in Davidson and McKey 1993; Heil and McKey 2003; and ant–fungi–bacteria in Poulsen and Currie 2006) and are featured in several books (e.g. Beattie 1985; Huxley 1991; Rico-Gray and Oliveira 2007; Stadler and Dixon 2008). We encourage readers to seek out these more in-depth works. Second, the mutualisms we describe often include currencies based on antagonistic interactions and/or access to food. Competition, predation, and parasitism of (and by) ants are treated in other chapters (see Chapters 5, 12, 9, 10, and 11, respectively, and Box 6.1), and aspects of ant diet and shelter are the focus of Chapter 7. In many cases, dissecting mutualistic interactions requires an understanding of those currencies.

We begin by describing mutualisms on the basis of the resources and services being traded. We focus on trophobiotic interactions (Section 6.2), wherein ants receive access to food resources in exchange for services provided to the reward producer (whether plant or insect; bacterial endosymbionts are discussed in Chapter 7), interactions where ants receive nutritive profit while dispersing plant propagules (seeds and pollen) (Section 6.3), and the tripartite mutualism among ants, fungal cultivars, and bacteria, in which food, protection, and dispersal are the currencies (Section 6.4). In each case, we identify instances in which these interactions can have consequences for the larger biotic communities and identify characteristics of ants that make them particularly well suited for participation in the interaction. We then take a synthetic approach to explore elements of context

Box 6.1 'Berry' ants: an eye-popping symbiosis from the rainforest canopy

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Successful transmission to a terminal host is one of the biggest challenges in a parasite's life cycle. Consequently, parasites have evolved a variety of mechanisms to change the behaviour and appearance of intermediate hosts to facilitate their consumption by the subsequent hosts. Several remarkable examples of this phenomenon involve ants as intermediate hosts, including the grass-climbing behaviour of *Formica* spp. infected with the fluke *Dicrocoelium dendriticum*, the yellow colour of cestode-infected *Leptothorax* spp., and the distended gasters of fluke-infected *Camponotus* spp. These and many other examples are summarized in reviews by Schmid-Hempel (1998) and Moore (2002). Evolutionarily, these changes in host appearance or behaviour are often interpreted as extended phenotypes of the parasites (Dawkins 1982; Hughes *et al.* 2008).

Recently, a striking case of ant manipulation by a parasite was uncovered in the rainforest canopies of Panama and Peru. Workers of the arboreal ant *Cephalotes atratus* infected by the nematode *Myrmeconema neotropicum* have red gasters containing several hundred worm eggs (Poinar and Yanoviak 2008; Yanoviak *et al.* 2008b). The life cycle of the nematode is closely linked to the life cycle and temporal polyethism of the ant; peak redness occurs when the ant is spending large amounts of time outside the nest, and coincides with the presence of infective nematode larvae within the eggs. The colour change is not caused by the deposition of red pigments. Rather, it results from localized exoskeletal thinning or leaching of pigments by the developing worms. This dramatic change in appearance is accompanied by continuous gaster-flagging and a substantially weakened postpetiole, characteristics not found in healthy ants. During the latter stages of infection, the parasitized ant becomes sluggish and assumes an erect posture (Plate 4).

In combination, these changes likely facilitate the consumption of ant gasters by frugivorous or omnivorous birds, which presumably mistake

the red gasters for ripe fruit (Yanoviak *et al.* 2008b). Unlike the examples mentioned earlier, ants are the final hosts for this parasite, and birds function as paratenic hosts (i.e. animals that transmit parasites to new hosts without becoming infected themselves; Moore 2002). Unfortunately, direct evidence for bird predation on infected gasters is lacking. However, given what is known of the natural history of *C. atratus* (especially their frequent foraging on bird faeces; reviewed by de Andrade and Baroni-Urbani 1999) and circumstantial evidence from field experiments (Yanoviak *et al.* 2008b), fruit mimicry remains the most parsimonious explanation. Many Neotropical angiosperms have small red fruits available at different times of year, and it is logical that a bird foraging on such fruits would sample any similar red object in its vicinity.

A plausible alternative hypothesis to fruit mimicry is that the red gasters make *C. atratus* workers more conspicuous to predators. Such 'increased conspicuousness' strategies are common among parasites, although few have been studied experimentally (Moore 2002). Increased conspicuousness is not supported in this case for at least two reasons. First, *C. atratus* is already one of the most conspicuous arboreal ant species in Neotropical lowland rainforests. Aside from non-selective foraging by tropidurid lizards, the workers are generally ignored by insectivorous vertebrates (de Andrade and Baroni-Urbani 1999; S. Yanoviak, personal observation). Thus, although infected workers stand out from healthy workers, this difference is unlikely to greatly increase predation on a common but unpalatable ant that is already an easy prey.

Second, the colour red is generally aposematic in insects. To overcome this strong negative signal, infected ants should resemble non-insects, or red gasters should provide a tasty reward. At the peak of infection (Plate 4), parasitized workers are practically immobile. They resemble ants morphologically, but not behaviourally. Given that nematode eggs pass through birds undigested (Yanoviak *et al.*

continues

Box 6.1 continued

2008), there is no obvious reward (nor penalty, excluding effort) associated with consuming an infected ant. Thus, a fundamental assumption of the increased conspicuousness hypothesis – that an attractive signal is associated with valuable resources – is not supported. Likewise, if there is no negative consequence of gaster consumption (a sting or noxious chemical), this mistake should persist in the bird's behavioural repertoire.

Symbioses between ants and other organisms are common and well documented;

ant–plant and ant–fungal mutualisms support entire research programs, books, and conference symposia. In contrast, ant symbioses with nematodes (Poinar *et al.* 2006) are under-investigated. Such parasitism is frequently overlooked or mistaken as a taxonomic variety, as occurred with red-gastered *C. atratus* over a century ago (Poinar and Yanoviak 2008). 'Berry' ants exemplify the remarkable interconnectedness of species in tropical forests, and hopefully will stimulate additional research on ant–parasite interactions.

dependency in these interactions (Section 6.5), and put this variation in the context of macroevolutionary variation (Section 6.6). Finally, we highlight the utility of these interactions for addressing questions fundamental to the field of ecology (Section 6.7) and conclude (Section 6.8) by identifying promising areas of future research.

6.2 Ants providing protection for food

Trophobiotic interactions involve the consumption of a food reward, often in return for protection from natural enemies. For ant-loving hemipterans, caterpillars, and most plants, these rewards almost invariably involve a sugary and/or nutrient-rich liquid, one that is collected by the foragers that patrol the area surrounding the resource (Plate 3). Highly specialized ant-plants (myrmecophytes) offer additional food rewards and provide ants with a domicile.

6.2.1 Sap-feeding hemipterans

Many ant species engage in mutualisms with herbivorous hemipterans (Stadler and Dixon 2005; Way 1963), and 41% of ant genera include trophobiotic species (Oliver *et al.* 2008). 'Myrmecophily' (ant-loving) occurs within most families of the Sternorrhyncha and Auchenorrhyncha (Hemiptera), including aphids (Aphididae), coccids and scales (Coccoidea), and membracids (Membracidae) (Sta-

adler and Dixon 2005). Within aphids, 40% of species are ant-tended, and many aphid genera include both tended and untended species (e.g. Mooney *et al.* 2008). These hemipterans tap into host plant phloem sap, which is rich in carbohydrates but relatively poor in nutrients and amino acids. As a consequence, sap-feeding hemipterans must dispose of large quantities of processed, but nonetheless sugar-rich, fluid. Many ants collect this sugary liquid waste, commonly referred to as honeydew. Ant attendance often results in larger hemipteran colonies (Way 1963) and greater fecundity (Bristow 1983; Del-Claro and Oliveira 2000). Ants that otherwise prey upon arthropods do not attack the sap-feeding herbivores, or at least do so more rarely. However, the incentives to view some proportion of an aphid colony as prospective prey rather than mutualistic partners may increase as honeydew-supplied carbohydrates become less limiting with colony growth (Cushman 1991; see Figure 6.1). In addition to this occasional predation, hemipterans may also bear yet unrevealed ecological or physiological costs from their mutualisms with ants. For example, in the absence of predators, ants can reduce aphid reproduction (Stadler and Dixon 1998, Yao *et al.* 2000).

The mechanism of ant benefits to tended hemipterans is most often presumed to occur via protection from natural enemies (Buckley and Gullan 1991; Stadler and Dixon 2005; Way 1963). Ants provide protection against enemies that are frequently

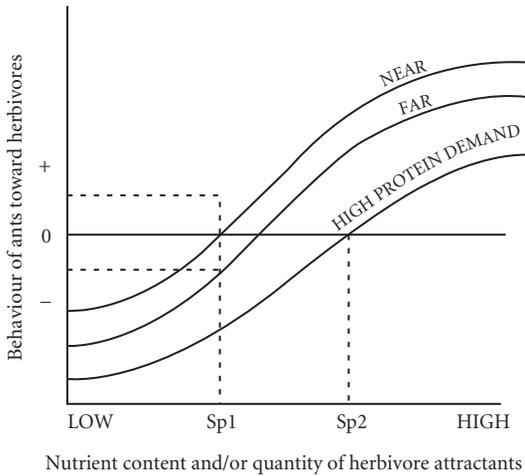


Figure 6.1 An ant's response to prospective mutualists may vary with the prospective benefits and costs of interacting with that partner. Cushman (1991) proposed that incentives for antagonistic interactions towards ant-tended herbivores (e.g. predation) will increase with travelling costs, the ant colonies' demand for protein, and where the quality or quantity of rewards offered by the prospective mutualists is low. When reward quality is high (e.g. Sp2 relative to Sp1), protein demand is low, and prospective partners are close to nests, ants are more likely to act as mutualists. (Reproduced with permission, from Cushman 1991).

somewhat specialized as hemipteran predators, including ladybird beetle larvae and adults (Coleoptera: Coccinellidae), syrphid fly larvae (Diptera: Syrphidae), lacewing larvae (Neuroptera: Chrysopidae), and parasitoid wasps (Hymenoptera: Braconidae), although ants can also provide protection against more generalist predators such as spiders (Cushman and Whitham 1989; Del-Claro and Oliveira 2000). Other benefits can include reduced fouling from honeydew accumulation (Bach 1991), reduced competition from other herbivorous insects (Smith *et al.* 2008), and allowing aphids to divert resources away from predator avoidance or parental care, and towards feeding, growth, and reproduction (Abbot *et al.* 2008; Bristow 1983; Flatt and Weisser 2000). Such non-protective benefits are rarely studied, and their frequency or importance compared to protection from predators is not well understood.

It would seem that the entire benefit of tending hemipterans for ants is nutritional. Aside from

water, carbohydrates are the dominant constituents of hemipteran honeydew. However, sugar type, nutrients, and plant secondary compounds all influence the attractiveness and presumably nutritive value of honeydew for ants (Blüthgen *et al.* 2004b; see Figure 6.1 and Chapter 7). Because direct measures of ant fitness are rare (but see Helms and Vinson 2008), we can only infer that honeydew attractiveness is indicative of nutritive value and value to the colony.

Because ants are effective and abundant predators of many arthropods, ant-hemipteran mutualisms have been defined as a 'keystone interaction' (Styrsky and Eubanks 2007) where variation in the strength or occurrence of the interaction has far-reaching consequences for the community in which it is embedded (see Bishop and Bristow 2001; Kaplan and Eubanks 2005; O'Dowd *et al.* 2003; Wimp and Whitham 2001). With respect to herbivores, hemipteran-tending ants increase mutualist abundance while often preying upon untended herbivores (e.g. Bishop and Bristow 2001; Mooney 2007). Key questions for the ecology of ant-hemipteran mutualisms have been whether the net effect of tending ants is to increase or decrease total herbivore abundance, and to what indirect effect on plant growth and fitness (e.g. Horvitz and Schemske 1984). Thus far, the literature suggests that hemipteran-tending ants typically depress the local abundance and species richness of several guilds of chewing herbivores, often to the host plant's benefit (Styrsky and Eubanks 2007). There are, however, spectacular exceptions to this generalization (e.g. Box 15.1; O'Dowd *et al.* 2003).

6.2.2 Lepidopterans

Approximately 70% of Lepidoptera in the family Lycaenidae (an estimated 6,000 species) whose life histories are known engage in associations with ants (Eastwood *et al.* 2006; Fiedler 2006; Pierce *et al.* 2002). Although some species of lycaenid may act as parasites of ants, we focus on the mutualistic species (Travassos and Pierce 2000). The lycaenid larvae and/or pupae attract the attention of ants by producing nitrogen-rich secretions (e.g. Agrawal and Fordyce 2000; Devries 1991), in some cases complemented by chemical and acoustic signalling

(Devries 1991; Travassos and Pierce 2000). Perhaps as a result of the nitrogen investment in reward production, species that feed on nitrogen-fixing plants or on nitrogen-rich plant parts such as flowers and seed pods are more likely to be ant-tended than are species that feed on other plant types or parts (Pierce 1986 but see Billick *et al.* 2005; Fiedler 1995). As with ant-tended hemipterans, ants benefit lycaenids by reducing attacks by parasitoids and predators (e.g. Devries 1991; Pierce and Mead 1981; Wagner and Kurina 2003). Lycaenids may add mass and reach maturity more quickly when protected by ants (Cushman *et al.* 1994), and some species preferentially oviposit in sites where ant densities are high (Wagner and Kurina 2003).

6.2.3 Extrafloral nectary-bearing plants

Plant species in over 90 families attract ants to nectaries not associated with flowers (hereafter, extrafloral nectaries or EFNs; Koptur 1992). These structures typically produce carbohydrate-rich nectar that can also include trace amounts of nitrogen or amino acids (Koptur 1992; Chapter 7). The nectar-attracted foragers may subsequently patrol the plant in search of further nectar, to deter competitors, and/or to consume alternative food items. This combination of foraging, deterrence, and consumption can decrease or alter the distribution of herbivory (Chamberlain and Holland 2009; Heil *et al.* 2001; Koptur 1992; Ness 2003a; Oliveira *et al.* 1999; Rudgers and Strauss 2004; and see appendices in Rico-Gray and Oliveira 2007), typically to the net benefit of most ant-tended plant partners (Chamberlain and Holland 2009).

The interaction between EFN-bearing plants and ants often includes multiple ant species sympatrically foraging on the same plants or plant species in the same population (e.g. Cuatle *et al.* 2005; Oliveira *et al.* 1999; Schemske 1980). These foraging bouts may be segregated in space or time (i.e. within particular branches, diurnal versus nocturnal, within particular temperature ranges or seasons; Díaz-Castelazo *et al.* 2004; Oliveira *et al.* 1999; Rico-Gray 1993; Schemske 1980). As a result, the EFNs on a particular plant may provide an important resource for an ant community even if it is not particularly important for any one population or colony (Blüth-

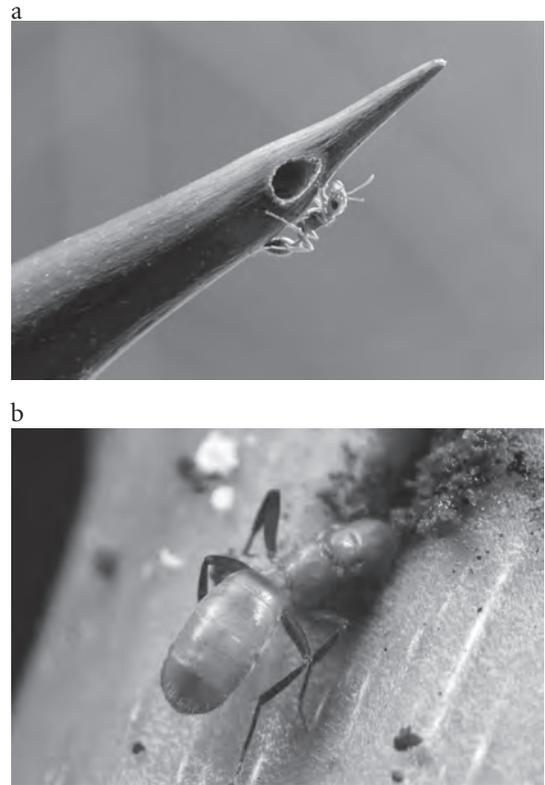


Figure 6.2 Myrmecophytes offer domatia in a variety of forms, many requiring that the ants gain access to a hollow structure by cutting through plant tissue. (a) A *Pseudomyrmex spinicola* worker at the entrance of its nest on a swollen thorn *Acacia*. The thorn is hollow but the ants must cut a hole to gain initial entrance. (b) An *Azteca isthmica* queen cutting into the soft tissue of a *Cecropia* tree to start a nest in the hollow center. (Photos: Alex Wild)

gen *et al.* 2004b; Díaz-Castelazo *et al.* 2004; Oliveira *et al.* 1999).

6.2.4 Myrmecophytes (ant-plants)

Plants in over 100 tropical genera host ants in specialized structures such as swollen thorns, hollow stems, and leaf pouches, collectively termed domatia (Bronstein *et al.* 2006; see Figure 6.2). These myrmecophytes or ‘ant-housing plants’ can also provision ants with lipid and protein-rich food bodies and/or nectar (Heil and McKey 2003; O’Dowd 1982), or host honeydew-producing

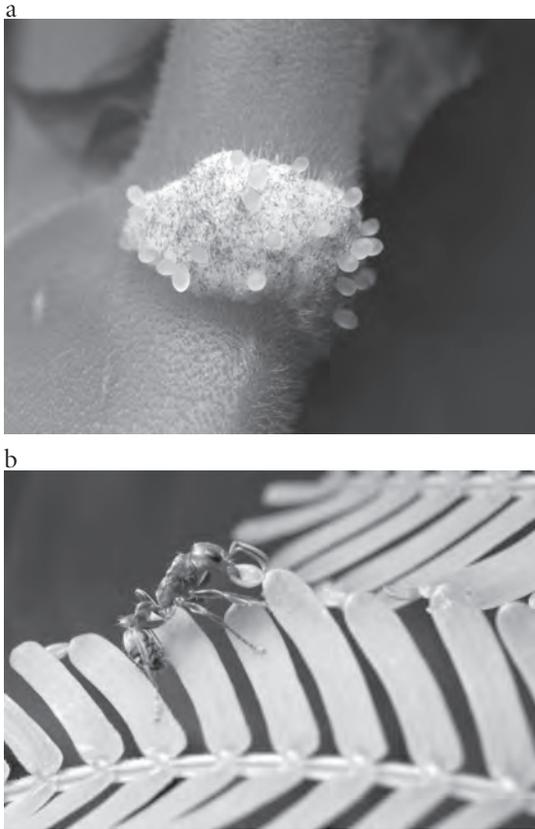


Figure 6.3 Myrmecophytes differ in the types of food they offer to their resident ants. (a) Lipid- and glyco-gen-rich Mullerian food bodies on a *Cecropia* tree. (b) A *Pseudomyrmex spinicola* worker on a swollen thorn *Acacia* harvesting a protein-rich food body to feed to the colony's larvae. (Photos: Alex Wild)

hemipterans (Gaume *et al.* 1998; Palmer *et al.* 2008) (see Figure 6.3). Some of the best-studied myrmecophytic relationships include those of *Acacia* with *Pseudomyrmex* ants (e.g. Janzen 1966, 1967a), *Cecropia* with *Azteca* spp. and other ants (e.g. Folgarait and Davidson 1994, 1995; Longino 1989), and *Piper* with *Pheidole* ants (Letourneau 1983; Letourneau *et al.* 2004) in the Neotropics, and *Macaranga* with *Crematogaster* ants in southeast Asia (e.g. Feldhaar *et al.* 2003; Fiala *et al.* 1989; Itino *et al.* 2001). Myrmecophytic *Acacia*, *Macaranga*, *Cecropia*, and *Piper* provide their ant partners with domatia and food bodies (see reviews in Davidson and McKey 1993; Rico-Gray and Oliveira 2007), whereas

myrmecophytic *Hirtella* spp. and *Tachigali myrmecophila* do not produce food rewards but instead support hemipterans that nourish the resident ant colonies (Rico-Gray and Oliveira 2007). Myrmecophytic interactions are much less widespread than facultative ant-plant associations involving EFNs (Heil and McKey 2003) and exhibit much greater specialization by both partners. For example, some myrmecophytic *Acacia* offer extrafloral nectar high in invertase (sucrose-cleaving enzyme) and low in sucrose, which corresponds to the preference of resident *Pseudomyrmex* ants for sucrose-free nectar that is unpalatable to other ants (Heil *et al.* 2005). Morphological adaptations include the prostoma (unlignified organ at the tip of the domatia) of *Leonardoxa* plants, the shape, and size of which corresponds strongly to the head of mutualistic ants (Brouat *et al.* 2001) and the wax crystals on the stems of some *Macaranga* that exclude ants not adapted to the slippery surface (Federle *et al.* 1997).

Plant-dwelling ants may provide nutrients, and/or protect their hosts from invertebrate and vertebrate herbivores, plant pathogens, and encroachment by competing plants (Bronstein *et al.* 2006; Davidson and McKey 1993; Heil and McKey 2003). These benefits can be pronounced. For example, a successful ant-plant symbiosis can create large monospecific 'devil's gardens' within otherwise diverse tropical rainforests (Frederickson *et al.* 2005). Plants that house ant residents may also (or instead) benefit from greater access to nitrogen and CO₂ as a result of the activities of its plant-dwelling ants (Sagers *et al.* 2000; Treseder *et al.* 1995). The benefits of its resource transfers may exceed the value of any protection provided by the ants in some systems and/or ecological settings.

6.2.5 The best ant partners

From the trophobiont's perspective, the ideal protectors are competitively dominant ants capable of aggressive behaviours (biting and stinging) and mass recruitment (e.g. Buckley and Gullan 1991) that might deter the partner's natural enemies. Ant characteristics that lessen the costs of foraging or patrolling can also increase the likelihood of mutualistic interactions by allowing a trophobiont that

produces modest rewards to, nonetheless, engage ants in a beneficial manner (see Figure 6.1). For example, foraging costs are reduced if the prospective ant partners are capable of establishing satellite nests at the base of plants with EFNs or hemipteran aggregations, or if the plant itself is the domicile of the colony. Because trophobiosis invariably involves the collection of sugary and/or nutrient-rich liquid, key adaptations include the capacity for trophallactic exchange of liquid food among members of the colony (Fiedler 2006) and morphological changes to the ant's proventriculus and gaster, which enable them to carry large amounts of sugary fluids (honeydew and nectar) and regulate the digestion of these fluids (Davidson *et al.* 2004). This is one explanation why subfamilies such as Formicinae and Dolichoderinae (and, more rarely, Myrmicinae), whose members have some subset of these adaptations, are the most common tenders, and why these foragers may tend to more than one of these partners within a particular habitat (e.g. Blüthgen *et al.* 2000; Devries 1991; DeVries and Baker 1989; Rico-Gray 1993).

From the perspective of the myrmecophyte, the ideal ant partner is quick to detect and deter would-be plant antagonists at a minimal cost to the plant. However, because the ecologies of myrmecophyte and plant-ant are largely inseparable, among-system transplants that could allow scientists to contrast the benefits of particular pairings are impossible. That is, we cannot test whether *Acacia* plants might do better hosting the *Azteca* ant associates of *Cecropia*. However, the diverging natural histories of particular systems provide clues to explain why the favoured (or at least realized) characteristics may differ among systems. Fast-growing pioneer trees with rapid rates of resource supply, such as *Acacia*, *Macaranga*, and *Cecropia*, often host an active, aggressive workforce of large ants (Davidson and McKey 1993). Smaller trees and shrubs, such as *Leonardoxa* and *Piper*, often host smaller, more timid or sluggish workers that can nonetheless be effective against very small herbivores, eggs, and microbes (Gaume *et al.* 1997; Letourneau 1983). Food (Itino *et al.* 2001) and nesting site (Fonseca 1993) resources impose limits to the hosting capacity of plants and imply a trade-off between the number and size of ants that can be hosted. If the

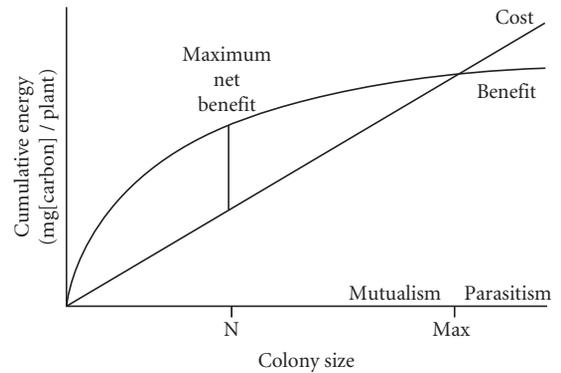


Figure 6.4 Graphic model of the cost and benefit to a myrmecochore of hosting an ant colony of varying size. The model describes a system wherein the cost of maintaining and housing ants increases linearly with colony size, while the benefits that ants provide saturate. *N* is the colony size that provides the greatest difference between benefit and cost (i.e., greatest net benefit) to the plant. Beyond a threshold ant colony size (*Max*), plant costs exceed plant benefit. Ant colonies at this stage may experience disproportional net benefit and/or be subject to plant reprisals. (Reproduced with permission, from Fonseca 1993).

incremental benefit to a plant of hosting additional ants progressively lessens as ant density increases and the structural or metabolic costs of hosting those ants increases linearly (i.e. each ant costs the same), the net benefit of hosting ants could lessen as colonies increase in size (Figure 6.4). This ant-plant conflict can influence the density of plant-ants that can occupy a given plant, population or community (Fonseca 1999), and may well dictate the best partner for particular settings.

The worst partner ants decrease the fitness of their partners. For example, in some settings, ants may consume more aphids than they protect from natural enemies (see Figure 6.1). Other costs might be more subtle. Highly aggressive ants that visit EFNs may also deter pollinators as effectively as they do to natural enemies (e.g. Ness 2006), leading to conflict between the defensive and reproductive mutualisms. Some ant residents also prune the flowers of their myrmecophytic hosts (Stanton *et al.* 1999; Yu and Pierce 1998) in an apparent effort to reallocate host resources towards ant rewards, enabling increased colony size at the plant's expense (see Figure 6.4). Ant-tended plants may



Figure 6.5 Ants from the genus *Rhytidoponera* are important seed dispersers. Here, *Rhytidoponera metallica* carries a seed with elaiosome attached. (Photo: Benoit Guénard)

limit these indirect costs by including ant-detering compounds in their flowers (e.g. Ness 2006; Willmer and Stone 1997a).

6.3 Ants provide dispersal for food

6.3.1 Seeds

Myrmecochory is the dispersal of ant-adapted seeds by ants. Over 90% of the >3,000 ant-dispersed plant species are found in the South African fynbos and in areas of Australia dominated by sclerophyllous plants (Berg 1975; Bond and Slingsby 1983). Most of the remaining identified ant-dispersed species are spring ephemerals in the temperate deciduous forests of northern Europe, Japan, and North America; myrmecochores account for 40% of the herbaceous species and 60% of emergent stems in portions of temperate deciduous forests of the eastern United States (Beattie and Culver 1981; Handel 1981).

Myrmecochorous seeds have an attached, lipid-rich food reward, called an elaiosome, which attracts ant foragers (Figure 6.5). Because the elaiosome's fatty acid composition is similar to that of insect prey (Hughes *et al.* 1994), the diaspore (seed + reward) is attractive to omnivorous foragers. Ants may preferentially collect seeds with larger elaiosomes or more favorable elaiosome-to-seed ratios (Mark and Oleson 1996), and some elaiosomes also include compounds that elicit collection behaviors by workers (e.g. 1,2-diolein in Hughes

et al. 1994). As a result of the nutritive value and chemical signalling component of the elaiosome and a durable seed coat, ants that might otherwise act as *seed* consumers are perhaps converted into *elaiosome* consumers, and hence, seed dispersers (Rico-Gray and Oliveira 2007). Interestingly, plants may also co-opt the attention of carnivorous ants; the most avid collectors of elaiosome-bearing seeds rarely include plant material in other aspects of their diet (Hughes *et al.* 1994). Myrmecochorous species are found in >80 plant families, and the morphological features associated with myrmecochory have evolved at least 20 times in the monocots (Dunn *et al.* 2007a). This estimate may be conservative, as some seeds that rely on ants for dispersal lack food rewards. For example, the diaspores of some 'ant garden' plants use odorants, rather than food rewards, as ant attractants (e.g. Youngsteadt *et al.* 2008). Whether that collection provides sufficient benefit to the ants to qualify as a mutualism is unclear.

The conventional forms of myrmecochory benefit the ant colony by providing a food resource that can enhance the colony's reproductive output (Gamans *et al.* 2005; Morales and Heithaus 1998). Myrmecochores produce their seeds in seasons where seed collection by ants is most likely to occur. This is the early summer in temperate deciduous forests, when ant foragers are both highly active and have dietary preferences that make elaiosomes attractive (Oberrath and Bohning-Gaese 2002), and plants have few opportunities for interactions with avian frugivores (Thompson 1981). The benefits to the seed include protection from granivores (e.g. Bond and Slingsby 1984; Christian 2001; Turnbull and Culver 1983) and/or fire (Christian 2001), directed dispersal to atypical microsites (such as nutrient-rich ant middens; Davidson and Morton 1981, but see Rice and Westoby 1986), dispersal away from parent plants and siblings (e.g. Bond and Slingsby 1984; Horvitz and Schemske 1986; Kalisz *et al.* 1999; Ness *et al.* 2004), and enhanced germination rates (e.g. Cuatle *et al.* 2005). The cumulative effect can be greater fitness for those seeds that are collected by ants (Hanzawa *et al.* 1988). Although these consequences are often studied in isolation, multiple benefits may be derived from any one ant-seed interaction (Giladi 2006).

6.3.2 The best ant partners

Changes in the seed-dispersing ant communities can alter seed survival rates, mean and maximum seed dispersal distances, and the distribution and composition of mature plant communities (Andersen and Morrison 1998; Bond and Slingsby 1984; Christian 2001; Ness *et al.* 2004; Ness and Morin 2008; Parr *et al.* 2007; Chapters 8 and 15). From the perspective of a myrmecochorous plant, ideal ant partners share several characteristics. High quality dispersers are typically solitary, omnivorous foragers that range far from their nest, disperse diaspores at substantial distances to those nests (the criteria for 'substantial' may be defined by the size of plants and the scale of soil heterogeneity within the site), feed on the elaiosome while leaving the seed intact, and bury the seeds shallowly in microsites where they can respond to germination cues (Giladi 2006). Low quality dispersers may be granivorous, disperse seeds at insufficient distances to avoid competition with maternal and sibling plants, dissect diaspores in situ (i.e. 'elaiosome robbing') rather than carry them to the nest, and cache high densities of seeds deep underground where germination is unlikely (Giladi 2006). Well-studied 'high quality' ants that collect a disproportionate amount of myrmecochorous seeds are *Rhytidoponera* spp. in Australia (Figure 6.5; Andersen and Morrison 1998; Gove *et al.* 2007; Hughes *et al.* 1994) and the *Aphaenogaster rudis* complex in North America (Beattie and Culver 1981; Ness and Morin 2008).

6.3.3 Pollen

The ubiquity of ants and their diverse interactions with plants begs the question of why ants so rarely act as pollinators. Several characteristics make ants poor candidates: maximum foraging distances are short relative to winged visitors, ant territoriality may decrease the likelihood of outcrossing among plants, and exposure to ants can reduce the viability of pollen (likely due to ant-borne antibiotics; Beattie *et al.* 1984). In so far as these shortcomings decrease the success of both male and female plant function (e.g. Galen and Butchart 2003), flower-visiting ants may be unwelcome 'parasites' of the interaction

between plants and legitimate pollinators. These consequences are the likely selection pressures for the chemical and physical impediments that can deter ants from entering flowers (e.g. Galen and Butchart 2003; Ness 2006).

Plant characteristics that can favour pollination by ants (or increase the incentives for ant pollination) include living in sites where ant activity is high (and/or other pollinators are rare), few synchronously blooming flowers per plant (to minimize intra-plant pollination or stigma-clogging for self-incompatible plants), pollen volumes insufficient to elicit grooming behaviours by the ants, and nectar rewards sufficiently unrewarding to discourage visitation by alternative, more expensive, pollinators (Hickman 1974). Although rare, such systems do exist. There are also a few plant species that receive pollination services by mimicking opportunities for ant copulation (e.g. *Leporella fimbriata* orchids are pollinated by male *Myrmecia urens*, Peckall 1989).

6.4 Ants, fungi, and bacteria

Originating 50 Mya (Schultz and Brady 2008), the tripartite association among ants, fungal cultivars, and actinomycete bacteria is perhaps the most highly evolved and complex set of mutualisms in ant ecology. More than 210 species in 13 genera of Myrmicine ants in the New World Attini tribe cultivate basidiomycete fungi as their main food source by collecting and preparing an appropriate fungiculture substrate (Currie 2001; Poulsen and Currie 2006). The ant genera vary in their choice of fungiculture substrate, colony size, and polymorphism. The more basal or 'lower' attines utilize insect corpses, faeces, or plant detritus as fungal-growing substrates, and tend towards smaller, monomorphic colonies. In contrast, the more derived or 'higher' attines utilize plant detritus or fresh plant material (e.g. leaf-cutting ants; Figure 6.6), can display extreme polymorphism, and may achieve colony sizes of several million individuals (Currie 2001; Poulsen and Currie 2006). Queens and larvae of attine ant colonies feed exclusively on the fungus, while workers may supplement their fungal diet with plant sap (Quinlan and Cherrett 1979). In the case of leaf-cutting ants, the fungi convert

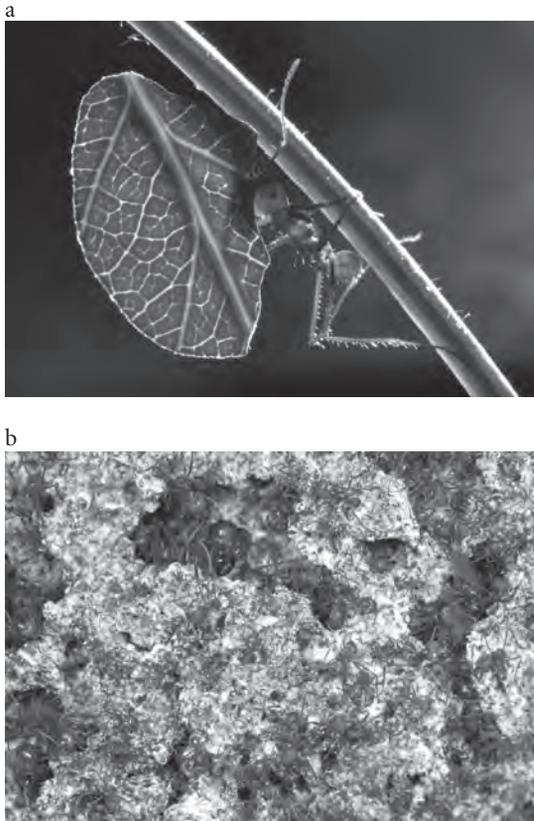


Figure 6.6 *Atta* are among the most conspicuous ants in the Neotropics and their colonies can number millions of workers in multiple subcastes. (a) An *Atta cephalotes* worker carries its harvest back to the nest to feed the colony's fungal cultivar. (b) *Atta cephalotes* workers tend the colony's fungal garden. (Photos: Alex Wild)

inedible plant material into lipid and carbohydrate-rich gonglydia, making the monophagous ants 'ecologically polyphagous' (Rico-Gray and Oliveira 2007). As a result, the ant–fungal composite feeds on a great diversity of widely distributed plants that would otherwise be inaccessible to the fungi and/or inedible to the ants.

Fungus-cultivating ants have an elaborate set of behaviours and traits that facilitate fungal cultivation. The selection of an appropriate substrate is key to fungal growth. Leaf-cutting ants avoid harvesting from plants with incompatible chemistry, possibly via feedback from the fungus (North *et al.* 1997). Attines further promote the growth of their fungal cultivars by pruning, redistributing fungus-pro-

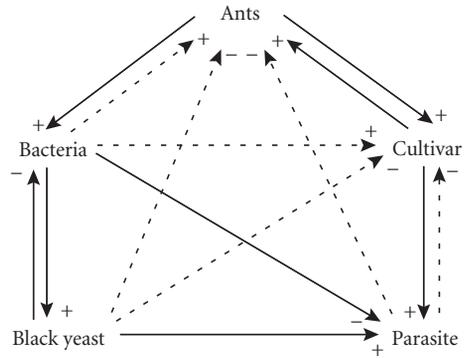


Figure 6.7 A diagram of the direct and indirect interactions of the attine ant-microbe symbiosis. Solid lines represent direct effects, dashed lines represent indirect effects, requiring the presence of an intermediary species. Cost (-) or benefit (+) deriving from the interaction is indicated at the tip of the arrowhead. Cultivar = fungal cultivar; parasite = specialised fungal parasite, *Escovopsis*; bacteria = actinomycete bacteria, *Pseudonocardia*, hosted on the ants; and black yeast = parasite of the bacteria. (Modified with permission, from Little and Currie 2008).

duced proteolytic enzymes around the fungal garden, and maintaining the garden chamber at the appropriate temperature and humidity (Poulsen and Currie 2006). The ants employ behavioural and chemical means to protect their fungal gardens from other microbes. The use of a platform by founding *Atta* queens reduces the risk of infection by microbes in the soil (Fernández-Marín *et al.* 2007). Weeding and grooming by workers also reduce contamination by non-mutualist microbes (Currie and Stuart 1991). Metapleural gland secretions provide effective general antibiotics and defend the fungal cultivars from an array of microbes (Poulsen *et al.* 2002; see Box 9.1).

The weeding and grooming behaviours and metapleural gland secretions are not effective against specialized fungal parasites in the genus *Escovopsis*. Thus the ants and their fungi depend on another mutualist, actinomycete bacteria (Figure 6.7). These actinomycetes, in the genus *Pseudonocardia*, are reared in specialized, elaborate crypts present in genus-specific locations on the cuticles of attines (Currie *et al.* 1999, 2006). The bacteria produce antibiotics that selectively inhibit the growth of *Escovopsis* (Currie *et al.* 1999) and are associated with all attine ants that have been examined (Currie *et al.*

2006). In *Acromyrmex*, the bacteria are most abundant on major workers that are most active at the bottom of the fungal garden where *Escovopsis* is most likely to be encountered (Poulsen *et al.* 2002). The actinomycetes further benefit the fungal cultivars by providing growth-promoting compounds (Currie *et al.* 1999) and may also directly benefit the ants by protecting them from pathogens (Currie 2001). Benefits conferred on *Pseudonocardia* by the ants include dispersal (by virgin queens during the nuptial flight), provision of a unique habitat in the cuticular crypts, and nourishment from specialized glands (Currie 2001; Currie *et al.* 2006). However, symbiotic black yeasts can parasitize *Pseudonocardia*, reducing its growth and decreasing the ability of the ants to suppress *Escovopsis* infection of their fungal gardens (Figure 6.7; Little and Currie 2008).

The synergism between leaf-cutting ants and leaf-digesting fungi provides perhaps the most dramatic example of the community-wide consequence of ant mutualisms. This ant-fungal collaboration exploits up to 50% of the plant species (Cherrett 1968, 1972) and can remove >10% of total leaf production in some Neotropical forests (Hölldobler and Wilson 1990). Few other herbivorous animals approach this breadth or magnitude of impact. Not surprisingly, this consumption can profoundly constrain plant recruitment and direct the nature and pace of plant succession (Vasconcelos and Cherrett 1997; Wirth *et al.* 2003), and the consolidation of resources in and around leafcutter nests can alter soil properties and the distribution of in-soil resources (Farji-Brener and Illes 2000; Wirth *et al.* 2003).

Fungiculture in non-attine ants is much less studied. Ants in the Old World *Lasius* genus, in the subgenera *Dendrolasius* and *Chthonolasius*, utilize ascomycete fungi to bind shredded wood or soil to reinforce nest walls. The ants nourish their fungi with honeydew and protect them from competing fungi, possibly through grazing (Schlick-Steiner *et al.* 2008).

6.5 Context dependency and stability

Because mutualisms are, by definition, reciprocally beneficial interactions, it is reasonable to ask what forces stabilize these interactions and regulate the

populations of the participants. That is, might the positive feedbacks derived from these interactions encourage these populations to grow progressively larger *ad infinitum*? At least three explanations clarify why this 'orgy of mutual benefaction' is so rarely observed (but see 'invasional meltdown' as in O'Dowd *et al.* 2003 and Box 15.1). First, other forces external to the mutualism, including natural enemies attracted by the success of one participant, intraspecific competition, or abiotic factors, may eventually limit the populations of at least one partner. For example, the black yeast symbionts that exploit the mutualism between fungus-growing ants and their actinomycete bacteria decrease the ability of the ants to protect their fungal gardens from the *Escovopsis* parasite (Little and Currie 2008), to the detriment of fungal garden health (Currie 2001). Second, the resources on which the interactions are based may collapse, as could be the case if a synergistic ant-aphid interaction was overly detrimental to the host plant. Third, the benefits conferred by participating in the interaction may saturate. For example, from the perspective of a plant or honeydew-producing aphid aggregation, the distinction between being tended by 5 ants versus 10 may be of negligible importance if 3 ants are sufficient to provide services required to increase their population (Ness *et al.* 2006). Additional ants may even be worse, if the costs of reward production increase linearly with partner abundance (Fonseca 1993; see Figure 6.4). Likewise, the carbohydrate rewards provided to these ants may become progressively less important to the well-being of the colony once access to protein, rather than carbohydrates, becomes limiting (see Figure 6.1).

Case studies of ants and their 'trophobionts' have shown remarkably disparate costs and benefits when studied in different settings. The benefits provided by ants to aphids have been shown to decline with aphid colony size (Breton and Adicott 1992). Because aphids can bear costs for provisioning mutualist ants (Stadler and Dixon 1998, Yao *et al.* 2000), the net benefit of ants can be predicted to depend on the risks from natural enemies, honeydew fouling, and competition from other herbivores. Moreover, aphids can compete intra- and interspecifically for ant attendance, and whether ants are mutualists for a particular aphid clone

depends on the availability of other sources of honeydew and nectar (Cushman and Addicott 1989). There is also evidence that host plants can mediate not only the strength of ant benefits to aphids, but also the direction of ant effects (Mooney and Agrawal 2008). Variation in phloem sap quantity or quality may be responsible for mediating these ant–aphid interactions (see also Figure 6.1).

Context-dependency might be particularly prevalent in interactions where ants primarily provide benefit by conferring protection. Most ant visitors (or at least visits) may not benefit the myrmecophile because: (a) the workers do not protect the partner (i.e. when ants are timid or ineffectual against ant-adapted herbivores) or (b) the partner's need for protection does not coincide with interactions with particular ant species or populations (e.g. EFNs: Schemske 1980; hemipterans: Cushman and Whitham 1989; myrmecochores: Fedriani *et al.* 2004). Perhaps as a result of selection pressures to increase the likelihood that ants can provide appropriate services when that service is required, the spatio-temporal distribution of reward production is correlated with the plant's vulnerability to natural enemies. For example, EFNs are often located in areas where the consequences of herbivory could be severe (e.g. young leaves, at the base of reproductive units; Horvitz and Schemske 1990; Oliveira *et al.* 1999; Schemske 1980). Likewise, myrmecochores drop seeds during the day, when foraging by granivorous rodents is lessened and the likelihood of seeds being collected by ants is greatest (Cuatle *et al.* 2005; Turnbull and Culver 1983).

If mutualist ants, or the subset of ants that are particularly effective, are a limited resource, prospective partners will compete for their services and a subset may suffer from decreased service. For example, experimental augmentations of membracid aggregations decreased overall tending rates by ants due to the decrease in the ratio of ants to membracid. The consequence of this decrease in service was a >90% decrease in the production of membracid adults (Cushman and Whitham 1991). A shortage of mutualists, or the disincentives of supporting partners when they are unnecessary, may explain why some myrmecophilic partners have adaptations to help them attract additional ant partners. Some of the methods employed to

promote additional ant attendance include greater production of extrafloral nectar (Heil *et al.* 2001; Ness 2003a), ant domiciles (Stanton *et al.* 1999), lycaenid food rewards (Agrawal and Fordyce 2000), or acoustical (Morales *et al.* 2008a) and chemical (Del-Claro and Oliveira 1996) signalling to ants during times of need.

Some level of constancy in partner identity, quality, and benefit is essential if local evolutionary specialization for particular mutualisms is to occur at the population level. Some myrmecophiles will occur in settings where interactions with ants are more necessary, and hence, more beneficial (Rudgers and Strauss 2004). Among-site variation that is stable through time could result in local evolution if the effective population size of partners is small relative to the area occupied by a particular partner taxon (Horvitz and Schemske 1990; Rudgers and Strauss 2004), whereas temporal variation can only lead to diffuse selection by the assemblage of mutualists ('the interaction') rather than particular partner species. There is some evidence that the identity of ants that act as prospective mutualists varies greatly among sites but can be more consistent over time within sites than are other mutualisms (e.g. see EFN tending ants versus pollinators in Horvitz and Schemske 1990). This may be due to the longevity (and immobility) of individual colonies, relative to some of their partners.

6.6 Macroevolutionary patterns in the face of variation

Although over half the ant subfamilies do not include species known to engage in mutualisms with trophobiotic insects, mature plants (EFN-bearing or myrmecophytic) or fungi, the incidence of all three interactions are positively correlated with one another among the remaining, vigorously mutualistic, subfamilies (Oliver *et al.* 2008). Within those subfamilies, however, mutualisms with trophobiotic insect or plants are negatively correlated with those with fungi at the genus level. One explanation is that ant lineages need to specialize in one type of mutualism when the adaptations for service or receiving benefit in one mutualism diverge from another. Specifically, the characters that favour

collecting fungal substrates and maintaining suitable fungal growing conditions in return for edible mycelia differ from those of aggressive defense and ingesting sugary secretions (Oliver *et al.* 2008).

Among facultative associations, there is evidence that the adaptations favouring myrmecophily are evolutionarily labile (i.e. can be acquired and/or lost at the species level of resolution). Hemipteran traits associated with ant tending include modification of honeydew chemical composition, aggregated feeding, longer proboscis length (Bristow 1991, Shingleton *et al.* 2005), and loss of defensive structures and predator avoidance behaviours (Stadler and Dixon 2005). A complete understanding of hemipteran adaptations to ant-tending is still forthcoming; some of the observed associations between traits and ant-tending are known from single hemipteran lineages, while associations among taxonomically disparate species do not distinguish between evolutionary convergence (as is presumed) and common ancestry. Nevertheless, that myrmecophily is not constrained to any single lineage suggests multiple origins and high lability for mutualism with ants. Consequently, many untended hemipteran species may be only a few evolutionary or ecological steps away from such mutualisms, and adaptations may be subtle. Among ants, adaptations that are correlated with, and perhaps favour, trophobiosis include a modified proventriculus, polygyny, and polydomy (Oliver *et al.* 2008).

Ant–myrmecochore interactions were not included in the aforementioned phylogenetic analyses. In so far as elaiosomes are dead insect analogues, their collection and utilization by ants may require little specialization or trade-offs with other mutualisms. Further, the repeated independent origins of myrmecochory (Dunn *et al.* 2007a) and diversity of elaiosome shapes, weights, histological origins, caloric and nutritional content, and manner of diaspore presentation in that guild imply great generalization by the plants. Nonetheless, the existing field observations demonstrate that two ant genera collect a majority of the myrmecochorous seeds in sclerophyllous Australia (*Rhytidoponera* spp., Figure 6.5; Gove *et al.* 2007) and temperate North America (*Aphaenogaster rudis* complex). Whether this constitutes ‘specialization’ by the myrmecochorous guild (to say nothing of coevolution) is unclear.

The strongest evidence of coevolution and ‘partner-filtering’ occurs in the interactions among myrmecophytes and their partners (see 6.2.4, Bronstein *et al.* 2006; Brouat *et al.* 2001; Federle *et al.* 1997; Heil *et al.* 2005; Janzen 1966) and between fungus-farming ants and their symbionts. All of the studied fungus-growing ants have phylogenetically specific modified exoskeletons for housing and feeding, for example, *Pseudonocardia* bacteria; closely related ant species lack these modifications (Currie *et al.* 2006 but see Kost *et al.* 2007). That all fungus-growing ants host a strain of *Pseudonocardia* (Currie *et al.* 2006), suggests that there is a yet-to-be-discovered mechanism for preventing establishment by other bacteria that may not act as a mutualist to the ant or the fungal cultivar (Kost *et al.* 2007). Similarly, although the ant–fungal cultivar relationship is now thought to be indicative of more diffuse, rather than pairwise, coevolution (Mikheyev *et al.* 2006), the incompatibility of alien fungal strains and hostile ant behaviour towards alien fungal fragments can prevent the introduction of competing fungal clones (Poulsen and Boomsma 2005).

6.7 Model interactions for ecology

Ant mutualisms have several characteristics that make them ‘model systems’ for addressing questions regarding mutualism and plant defense. We highlight these advantages later, and propose promising research questions in Section 6.8.

First, ant attendance and behaviour can be monitored in real time. Ants that forage on the surface of plants, leaf litter, and soil can be counted. As a result, variation in the number or behaviours of foragers allocated to a particular task and turnover in the species performing a task can be accurately described. These measures can provide information about the costs and benefits received by each participant in the prospective mutualism, and how these vary over time, space, or in response to experimental treatments.

Second, ants can be excluded from particular microsites. Many studies of ant protection mutualisms use sticky substances (e.g. Tanglefoot™) to experimentally exclude ants from some subset of their partners, or from portions of particular partners (e.g. control versus treatment branches). Remarkably,

these exclusion treatments can even be performed at the scale of hectares (e.g. poison baits in Abbott and Green 2007). Ant densities can also be depressed by adding ant predators (e.g. Letourneau *et al.* 2004).

Third, many individual partners interact with one ant colony for sustained periods of time. This largely occurs as a result of a combination of the central-place foraging requirements of a (largely) immobile ant colony and territorial interactions between colonies and/or species. Although there are many exceptions to this gross generalization and the very definition of 'sustained' will depend on the lifespan of the partner, some ant mutualisms are believed to have continuously functioned for centuries (Frederickson *et al.* 2005). Irrespective of duration, this dynamic of sustained interactions between two individuals, or at least one individual and one superorganism, is more common in ant mutualisms than in those involving more mobile partners, such as pollination.

These three attributes of ant mutualisms have facilitated much of our understanding of ant and non-ant mutualisms (Bronstein 1998; Heil and McKey 2003), as well as plant defense, food web structure, and the dynamics of symbiotic interactions. For example, an ecologist's ability to reliably count and exclude ants (or ant-occupied thorns) from particular branches makes it possible to quantify and manipulate plant defenses to an extent that is nearly impossible (or at least terribly expensive) for chemical plant defenses such as tannins or alkaloids.

6.8 Future directions

In the following text, we highlight several ecological topics that we perceive as particularly critical and promising for better understanding the role of ants as mutualists.

6.8.1 Diverse partners

What are the consequences of interacting with a variety of prospective ant partners? There are many examples of great variation in partner quality (e.g. Buckley and Gullan 1991; Horvitz and Schemske 1986; Miller 2007b; Ness *et al.* 2004; Ness *et al.* 2006), and striking examples where a greater frequency of interactions with suboptimal partners lessens the benefits to the ant's partner (Christian

2001; Palmer *et al.* 2008). Nonetheless, how often interactions with suboptimal partners constrain selection for the mutualism is unclear.

Can the inclusion of suboptimal partners be beneficial, and are there settings in which the diversity of a partner assemblage itself confers benefits? For myrmecochores, a more diverse disperser assemblage could increase the variety of sites where seeds are deposited and, perhaps as a result, decrease the influence of detrimental density-dependent processes. The synergistic effect of multiple predators is well documented in other systems (Cardinale *et al.* 2003; Sih *et al.* 1998); that it occurs in ant protection mutualisms is a reasonable (Beattie 1985; Rico-Gray and Oliveira 2007), albeit largely untested, hypothesis. Further, repeated interactions with suboptimal partners, or interactions with many of those partners, can remedy the mediocrity that may be so pronounced on a per capita or per interaction basis (Ness *et al.* 2006). If partner diversity does confer benefits, the costs of participating in a mutualism that typically includes a diverse assemblage may only become apparent when a prospective mutualist is simultaneously deprived of those diverse partner assemblages and limited to interacting with one or a few partners that are mediocre (or outright parasitic) in all settings. That combination of homogeneity and inadequacy may be a historically rare phenomenon in the natural world. Or, put differently, myrmecophilic organisms may be rare in habitats where such conditions are the norm in the natural world. We predict such pairings may occur increasingly frequently due to anthropogenically induced disruptions in ant faunas in the face of landscape conversion, global climate change, and exotic ant invasions.

Partner diversity and specificity of the ant-fungi-bacteria mutualism are also ongoing subjects of investigation. Recent discoveries of filamentous actinomycete bacteria on non-attine ants that also inhibit *Escovopsis* growth have called into question the specificity of the attine ant-actinomycete mutualism and whether ants have any mechanism to control bacteria on their exoskeletons (Kost *et al.* 2007). In addition, much more is to be learned about the non-attine ants that cultivate fungi for architectural purposes. Is there a similar complex interplay of mutualists and parasites as has become evident in the attine ant fungal gardens?

6.8.2 Benefits to ants that engage in mutualisms

The vast majority of studies that explore the interactions between prospective mutualists have focused almost exclusively on the consequences of the interaction for the non-ant partner. This discrepancy may be partly attributable to (a) the logistical difficulties of measuring ant fitness (but see Cushman *et al.* 1994; Lach *et al.* 2009; Morales and Heithaus 1998); (b) the assumption that the participation of the more mobile participant (ant) is evidence of choice, and thus, benefit to that participant; and (c) the ease of quantifying other variables relevant to the ant's partner (e.g. defoliation, aphid mortality). Ant-fungal mutualisms are an exception; microbe partners or substrates can be manipulated, and outcomes for colony growth or survival can be measured relatively easily (e.g. Fernández-Marín *et al.* 2007; Seal and Tschinkel 2007a). One solution to this widespread shortcoming is to utilize the modular organization of ant colonies, and to more fully describe the effects of these interactions on individual modules (i.e. individual ants, see Cushman *et al.* 1994; Lach *et al.* 2009). Stable isotope techniques are one new promising technique to quantify the benefits that ants receive (e.g. Sagers *et al.* 2000; Box 7.1). Davidson *et al.* (2003) used stable isotopes to infer that access to extrafloral nectar and hemipteran exudates in the rainforest canopy fuel the spectacular diversity and abundance of ants in those habitats. Critically, stable isotope techniques highlight the integration of rewards into ant tissues or particular castes rather than measuring fitness, and their correct interpretation requires a comprehensive knowledge of the natural history of the system.

Barring obligate ant–myrmecophyte or ant–fungal interactions, we know of no studies that have sought evidence (much less demonstrated) that among-site variation in ant communities is attributable to variation in the availability of their mutualists (but see Díaz-Castelazo *et al.* 2004; O'Dowd *et al.* 2003). The inherent asymmetry in many ant–mutualist interactions offers one explanation for that absence. Ants often provide their plant and trophobiont partners with protection at critical demographic stages (e.g. protection of seeds and

aphid nymphs from predators), and they often literally transport those partners into their 'sphere of influence' (e.g. carrying seeds and aphids closer to the nest). In contrast, the benefits to the ants often are limited to the augmentation of resources that are already found in the regular diet of the ant.

6.8.3 Costs and cheating

To answer whether the net effect of an interaction is beneficial, our interpretation of the spatio-temporal heterogeneity in benefits should be balanced by an appreciation for the magnitude and variability of the costs of participating in the interactions. In so far as the benefit accrued by one partner translates into the cost experienced by the other, conflicts of interest between ants and their prospective mutualists may seem unavoidable (e.g. Section 6.2.5 and Figure 6.4, see also Palmer *et al.* 2008; Stanton *et al.* 1999; Yu and Pierce 1998). However, three largely untested hypotheses explain why this need not be the case:

The resources or strategies that benefit one participant may come at negligible cost to its partner. In such situations, the benefit to the recipient may not come at a commensurate cost to the provider, and one can get 'something for nothing'. The costs of plant-produced rewards can be quite minor (O'Dowd 1979, 1980), and are lessened when plants decrease or curtail extrafloral nectar production in the absence of perceived threats (Lach *et al.* 2009; Ness 2003a). Indeed, the multiple prospective benefits of ant attendance to hemipterans beg the question, why do not all hemipteran species exchange their waste products for ant attendance? Similarly, some of the benefits ants provide likely incur negligible cost to the colony. For example, some plants benefit from access to the debris accumulated by foraging ants and the CO₂ they exhale (e.g. Sagers *et al.* 2000; Treseder *et al.* 1995; Wagner 1997). Last, although participation in particular mutualisms may incur costs, those solutions are often cheaper than the alternatives (e.g. myrmecochory versus frugivory in nutrient-poor habitats; Westoby *et al.* 1991b).

The resources traded in these interactions may be less important than are other components of the interaction. For example, many ant-collected seeds

include odorants that elicit collection behaviours by workers (e.g. Hughes *et al.* 1994), and in some cases an elaiosome reward is entirely absent (e.g. Youngsteadt *et al.* 2008). In some respects, these interactions may function more as 'behavioural usurpation' than a reciprocal exchange of resources. Ecologists have yet to ask whether diaspores will be collected when these compounds are experimentally disassociated with the seed, although we know that other non-rewarding substances impregnated with the volatiles will be collected by workers (e.g. Hughes *et al.* 1994).

The outcome that benefits the myrmecophile may be a product of strategies that best suit the ant. In such a case, the concept of 'cheating' becomes meaningless. For example, if an ant colony is capable of the vigorous defence of a resource against real or perceived competitors, be they rival colonies, herbivores, or carnivores, it will do so. If it cannot, the opportunity to harvest that resource may well be usurped by a more aggressive colony that provides even greater protection to the reward (plant or insect). On a different vein, Ness *et al.* (2009) demonstrated that sustained collection of carbohydrate-rich resources changes ant dietary preference, and inferred that an abundance of one resource highlights the relative absence of complementary resources (here, provision of abundant carbohydrates elicit attacks on relatively nitrogen-rich prey). Last, from a myrmecochore's perspective, the most important characteristic of an ant is that it does not 'cheat' by removing the elaiosome and abandoning the denuded seed (to predators, competitors, etc). For subordinate ants that specialize in discovering but not dominating resources, the most advantageous behaviour may be to immediately collect the elaiosome (with the seed attached) rather than engaging in the time-consuming task of separating reward from seed, and hence risk losing the resource to a competitor.

6.8.4 Inter-mutualism conflict

Relatively little is known of the interactions between mutualisms, including those in which ants are involved. Such inter-mutualism dynamics may

play a central role in shaping the ecology and evolution of ant-mutualist interactions. For example, it has been proposed that EFNs have evolved as a means of distracting ants from tending hemipterans (Becerra and Venable 1989) and collecting floral nectar (Wagner and Kay 2002). However, ant-tended insects are disproportionately common on EFN-bearing plants (Offenberg 2000), and some even ingest extrafloral nectar (DeVries and Baker 1989). The rewards provided by hemipterans can also supplement the ant rewards provided by myrmecophytes (Fonseca 1993; Palmer *et al.* 2008). In so far as ants have greater control over hemipteran densities than they do direct plant rewards, the involvement of these third parties can affect the functioning of the symbiosis (Gaume *et al.* 1998).

Some plants also utilize ants within the context of multiple mutualisms involving protection and seed dispersal (*Turnera ulmifolia*: Cuatle *et al.* 2005; *Urera baccifera*: Dutra *et al.* 2006). The most thoroughly studied of these systems, and perhaps the most reticulate, is *Calathea ovandensis*; this tropical herb has EFNs, is attacked by ant-tended Lepidoptera, and relies on myrmecochory for seed dispersal (Horvitz and Schemske 1984, 1986). How often the coterie that participates in one interaction is well suited for the other, or interacts with that counterpart, is unknown (but see Cuatle *et al.* 2005). Explorations of these inter-mutualism dynamics may provide important insight into the evolution of ant mutualisms generally.

The multiple mutualisms and complex interactions occurring within the nests of fungal gardening ants are a rich area for exploring potentially competing mutualisms and the effects of parasites. The mutualism between actinomycete bacteria and ants and the parasitic black yeast-actinomycete bacteria and *Escovopsis*-fungal cultivar relationships have only recently been discovered to substantially shape the dynamics of the ant-fungal cultivar mutualism. Questions remain about the mechanisms through which some effects are seen. For example, given that actinomycete bacteria are stimulated by the presence of *Escovopsis* (Currie *et al.* 2003), are black yeasts as well? And if so, are there feedback mechanisms by which black yeasts facilitate *Escovopsis* infection? It is likely that new microbes that may further affect the costs

and benefits of the multiguild relationships await discovery.

6.8.5 Biotic interactions on an abiotic stage

Experimental studies have begun to explore the importance of variation in abiotic resources on the evolution and functioning of ant mutualisms. In so far as these resources are limiting, they can alter the incentives for particular interactions. For example, carbon-rich resources such as extrafloral nectar and ant domatia should be less costly for plants to produce where carbon is in excess (Folgarait and Davidson 1994). Perhaps as a result, EFN-bearing plants are common in sunlight-rich habitats such as rainforest canopies (Blüthgen *et al.* 2000), forest edges (Bentley 1976), and deserts (Pemberton 1988). The influence of resource limitation (or surplus) is also detectable at smaller spatio-temporal scales. Nitrogen fertilization of host plants can increase tending rates of some trophobionts (e.g. Lycaenids: Billick *et al.* 2005, but see Morales and Beal 2006 re. membracids), and alter plant investment in indirect defences (Folgarait and Davidson 1995). Ant mutualisms can also reorganize abiotic resources. The construction, maintenance, and feeding of ant colonies and nests often concentrate resources, expose buried nutrients, and alter moisture retention rates (Moutinho *et al.* 2003), perhaps to the benefit of their partners (Davidson and Morton 1981; Giladi 2006; Wagner 1997). Remarkably, there is also evidence that tending by ants can alter the nitrogen content of tended hemipterans and their host plants (Kay *et al.* 2004, but see Abbot *et al.* 2008). The generality of these documented patterns, and how such modifications will influence the incentives for ant mutualisms, is largely unknown. To make matters more complex (and worthy of attention), the availability of nitrogen and CO₂ continues to increase at scales ranging from individual plants to the biosphere as a result of anthropogenic influences.

6.8.6 Putting ant mutualisms in their place

Ant mutualisms are unevenly distributed across habitats. Perhaps problematically, the settings for the research that underpins our understanding of

these interactions rarely occur in proportion to the density or diversity of these interactions. How might our understanding of these interactions change if we studied them in the settings where they most often occur? Most studies of myrmecochory (and all that quantify benefit to the ants) focus on temperate deciduous myrmecochores; what do the costs and benefits described in this nutrient-, moisture-, and granivore-rich biome tell us about the >90% of myrmecochores that reside in dissimilar biomes in Australia and South Africa? Might our sense of the costs, benefits, and selection pressures on ant-tended insects and plants differ if we studied them in communities such as tropical rainforest canopies or some deserts where tending by ants is the modal interaction? The characteristics of ants, prospective natural enemies, and competition for services may be sufficiently different in these settings to profoundly alter those interactions. We recognize that particular systems offer advantages for studying particular ecological and/or evolutionary phenomena. However, we propose that the wealth of studies that comprise the current literature and inform reviews, meta-analyses, and our gestalt sense of how interactions function may describe the range and modal version of the interactions only in so far as those studies occur in comparable environments. Our understanding of those interactions will change as we better place our questions in the context of the larger environment.

6.9 Summary

Ants are perhaps the most common and dominant animal mutualists in terrestrial environments. As a result, better understanding the dynamics of these interactions should be a priority for those who hope to understand the taxon, their role in communities, and mutualism as a widespread interspecific interaction. These mutualisms include interactions with ant-loving plants, insects, fungi, and bacteria, with the ants typically receiving food and/or shelter, and their partners receiving food, protection, and/or propagule dispersal. Context dependency, wherein the magnitude of costs and benefits incurred as a result of participation in the interactions varies with the ecological setting may be particularly prevalent

in interactions involving protection by ants. Adaptations that enable effective participation in one type of mutualistic interaction may preclude a species from participating in others, but may also make it more difficult for non-beneficial interactors to intrude. Because ant interactions with their mutualists are relatively easily monitored, manipulated, and are sustained over time, ant mutualisms are model systems for understanding mutualisms and plant defence. We encourage future work that explores the influence of partner diversity, better quantifies

costs and benefits to participants, and addresses how contemporary interactions and abiotic resources alter these interactions.

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