

Interactions between ants and aphidophagous and coccidophagous ladybirds

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Introduction

Insects that are associated with ants are called myrmecophilous (from the Greek *myrmex*, meaning ant). Myrmecophilous aphids and coccids show behavioural and structural modifications to life with ants. When an ant encounters such an insect, it usually strokes it with its antennae. This induces the aphid or coccid to suppress its usual defensive behaviour of kicking out, running away, dropping off the plant, or clamping down. Instead it raises its abdomen and exudes droplets of honeydew from its cornicles, which the ants then imbibe. Ants gain food from the association, for honeydew is rich in carbohydrates and also contains amino acids, amides, proteins, minerals and B-vitamins (Way 1963; Carroll and Janzen 1973; Hölldobler and Wilson 1990). Under some conditions ants may also gain protein, by preying on the aphids or coccids.

The benefits of the association to the aphids or coccids include improved hygiene, through the removal of caste skins, dead aphids and honeydew (Way 1954; Banks 1958; Seibert 1992), direct increases in development rate, adult body size, fecundity and reproductive rate (El-Ziady and Kennedy 1956; Banks 1958; El-Ziady 1960) and protection from predators, parasitoids and parasites (Bartlett 1961; Banks 1962; Jiggins *et al.* 1993). Additional benefits may accrue because ants have been reported to transport aphids to new plants when old ones deteriorate, and ants may reduce competition by removing non-myrmecophilous aphids and other phytophagous insects (Majerus 1994).

By protecting homopterans from aphidophages and coccidophages, whether these are predators or parasitoids, ants come into conflict with such species (Dixon 1985; Rosen 1990). Some evolutionary and ecological responses of parasitoid Hymenoptera to this antagonism from ants have been demonstrated, including more rapid ovipositing (Bartlett 1961), avoidance behaviour (Völkl 1997) and a variety of chemical adaptations (Liepert and Dettner 1996; Völkl 1997). Less attention has been paid to the evolutionary and ecological responses of homopteran predators to ant attendance of their prey. Although a diverse array of attributes of aphidophages and coccidophages have been suggested to be the outcome of interactions with Homoptera-tending ants, the evidence supporting such suggestions is sparse, scattered back over more than a century, and is sometimes contradictory.

We here briefly review the literature relating to interactions between one group of homopteran predators, the ladybirds (Coccinellidae), and ants. We consider when and why ladybirds try to feed on ant-tended aphids or coccids as food, describe two case studies of myrmecophilous coccinellids and speculate on the evolution of responses to ants and the evolution of myrmecophily.

Ladybird-ant interactions

Interactions between ants and ladybirds may be of three types. First, ants that tend Homoptera will be in competition with aphidophagous or coccidophagous ladybirds for resources. Second, ladybirds may feed directly on ants, although only one species of ladybird is known to specialize on ants (Harris 1921). Third, ants may prey on ladybirds. The most useful division of these interactions is into those that are competitive and those that are non-competitive.

Non-competitive interactions

Non-competitive interactions may broadly be seen to include all those away from ant-attended homopteran colonies, plus those involving predation of ladybirds by ants (or the reverse). Such interactions are important because they can influence habitat preferences and the distributions of ladybirds within an environment. Away from homopteran colonies, ants that encounter ladybirds either attack them or ignore them, with the distinction between the two often depending on the species of ant. Thus, several species that attack coccinellids in the vicinity of tended homopteran colonies, including *Lasius niger* (El-Ziady and Kennedy 1956; Banks 1962), *Formica fusca* (Rathcke *et al.* 1967), *Iridomyrmex humilis* (Dechene 1970) and *Myrmica ruginodis* (Jiggins *et al.* 1993), are indifferent to ladybirds elsewhere. Conversely, some species of ant that are predatory on insects will attack ladybirds whenever they encounter them, and in consequence can exclude many species of coccinellid almost entirely from their forage range. Examples are difficult to cite for two reasons. First, most of the empirical evidence is based on introducing coccinellids to captive starved ant colonies, and interactions observed do not reflect what happens in the field. Thus, for example, Hays and Hays (1958) found that captive, starved *Solenopsis invicta* would kill and consume five species of coccinellid, yet Wilson and Oliver (1969) found only one coccinellid among 4056 prey items taken

by this ant in the field. Furthermore, Sterling *et al.* (1979) found that *S. invicta* presence did not reduce coccinellid numbers in cotton fields. Second, it is often difficult to disentangle the effects of ant predation of coccinellids and ant attendance of Homoptera on coccinellid distributions. *Oecophylla longinoda* has been recorded preying upon ladybird larvae and pupae (Mariau and Julia 1977). *Formica rufa* has been recorded killing and carrying larvae and adults of several coccinellids (Sloggett *et al.* 1999) while others have shown that *F. rufa* attack most coccinellids placed on their nests (Donisthorpe 1919-1920; Jiggins *et al.* 1993), and significantly reduces the density of coccinellids in its forage area (Majerus 1989; Sloggett 1998). However, the distribution of *F. rufa* within a habitat is far more influenced by the distribution of the aphids that it tends than it is by the distribution of insect prey (Whittaker 1991). Thus the differentiation of ant-coccinellid interactions into competitive and non-competitive may be overly simplistic.

Despite the artificiality of the divide of ant-coccinellid interactions into competitive and non-competitive, it is likely that highly aggressive predatory ants do impose a greater effect on coccinellid distributions in a habitat than do those ant species that only attack ladybirds close to or on homopteran colonies. Thus it is notable that *S. invicta*, which does not prey on coccinellids, but does exclude them from tended colonies of the cotton aphid *Aphis gossypii*, does not affect the large scale distribution of ladybirds in cotton fields (Sterling *et al.* 1979), in the way that *O. longinoda* and *F. rufa* influence coccinellid distributions within their habitats (Mariau and Julia 1977; Majerus 1989; Sloggett 1998).

Competitive ant-ladybird interactions involving homopterans

There is considerable observational and experimental evidence that Homoptera-tending ants show greater aggression towards coccinellids in the vicinity of tended colonies than elsewhere. This has been described as ownership behaviour (Way 1963). Aggression is shown towards both adult and larval coccinellids. Adults are usually chased from homopteran colonies (El-Ziady and Kennedy 1956; Dechene 1970; Bradley 1973; McLain 1980; Itioka and Inoue 1996; Sloggett 1998), while soft bodied larvae may be picked up and carried away from the colony, or dropped off the plant, or killed (El-Ziady and Kennedy 1956; Cochereau 1969; Bradley 1973; Vinson and Scarborough 1989; Bach 1991; Jiggins *et al.* 1993; Sloggett 1998).

Studies that have compared the densities of ladybirds in the presence and absence of ants have usually shown that ants do reduce the numbers of ladybirds on ant-tended colonies of both aphids and coccids. This is the case in studies in which the homopteran colonies being compared are naturally tended or untended by ants (Mariau and Julia 1977; McLain 1980; Völkl and Vohland 1996), or where untended colonies have been produced by preventing ant attendance by the use either of barriers (Bradley 1973; Reimer *et al.* 1993; Itioka and Inoue 1996), or by poisoning (Mariau and Julia 1973; Jutsum *et al.* 1981). Exclusion of coccinellids from homopteran colonies by ants has been shown to be beneficial to both tended aphids (Banks 1962; Mariau and Julia 1977; Kreiter and Ipert 1986; Reimer *et al.* 1993) and coccids (Bradley 1973).

Coccinellid predation of ant-tended Homoptera

A few coccinellids are considered to be myrmecophilous. While such species habitually live close to ant nests, non-myrmecophilous coccinellids only feed on ant-tended Homoptera when untended Homoptera are scarce (Sloggett 1998). That said, differences in the size, aggressiveness and density of tending ants, and in the size, behaviour and defensive capabilities of coccinellids, undoubtedly affect the level and outcome of interactions between ladybirds and ants.

Before considering predation of ant-tended Homoptera by coccinellids, it should be emphasized that the levels of predation of ant-tended aphids and ant-tended coccids by ladybirds might be quite different. As most coccinellids mainly attack ant-tended Homoptera when non-tended Homoptera are scarce, the probability of finding untended Homoptera becomes critical. Several factors may cause differences in the likelihood that coccinellids will encounter ant-tended aphids compared to ant-tended coccids. First, at higher latitudes, aphids are much commoner relative to coccids than in the tropics. Ant diversity and abundance is much greater in the sub-tropics and tropics than in more temperate climes. This leads to the suggestion that untended colonies of coccids will be less common than untended colonies of aphids. Second, and conversely, aphids are renowned for the ephemerality of their colonies, particularly in seasonal climes: coccids less so (Dixon 2000). In part aphid ephemerality is due to eradication of colonies by predator and parasitoid pressure. The result of the 'boom and bust' cycle seen in aphids is that their predators frequently face a dearth of aphids other than those that have been ant-tended and so have been protected from this predation/parasitoid pressure. Ant-tended colonies of a variety of species of aphid have been shown to persist for longer than untended colonies of the same species (Addicott 1979; Bristow 1984; Mahdi and Whittaker 1993; Sloggett 1998). Forage theory predicts that aphidophagous coccinellids are most likely to feed on ant-tended aphids when untended aphids are scarce – i.e. when untended aphids are of greater relative value (Stephens and Krebs 1986). In temperate regions untended aphids are often scarce in late summer, as a

consequence of both the production of dispersive alate forms, and predation and parasitoid pressure earlier in the summer. Conversely, ant-attended colonies often remain abundant at this time (Mahdi and Whittaker 1993; Sloggett and Majerus 2000a).

It is not clear whether overall aphidophagous coccinellids or coccidophagous coccinellids are under greater pressure to feed on ant-tended prey. What is clear is that the pressure will vary both with coccinellid species and with prey species. Moreover, this pressure will vary greatly in seasonal habitats (*sensu* Southwood 1977), which will be more pronounced in temperate regions than in the tropics. Work on the relative availabilities of untended and tended aphids and coccids through the year in a variety of climate zones is urgently needed, and may shed light on both similarities and differences in the interactions between coccidophagous and aphidophagous coccinellids and Homoptera-tending ants.

The most detailed work on coccinellid predation of tended and untended Homoptera has been conducted in temperate regions, with aphidophagous species. Aphidophagous coccinellids usually breed during periods of aphid abundance, when adults are feeding on untended prey (Majerus 1994; Hodek 1996; Sloggett 1998). Consequently, the immature stages of most coccinellids, with the exception of myrmecophilous species, will rarely come into conflict with ants tending aphids. Only in rare years when there is a general scarcity of aphids, are larval stages likely to try to attack ant-tended aphids, and so be under selection for adaptations that will enable them to feed on such prey (Sloggett 1998).

In contrast, adult coccinellids come into conflict with aphid-tending ants annually in late summer when feeding up prior to overwintering. Due to aphid scarcity, they feed at this time on a variety of foods, such as pollen, nectar, sap, honeydew, non-homopteran invertebrates, and conspecifics (Clausen 1940; Ipert 1965; Majerus 1994; Hodek 1996). Some also attack ant-tended aphids (Majerus 1994; Sloggett 1998; Sloggett and Majerus 2000a, 2000b). The tolerance of adult ladybirds to ant attacks then becomes a critical issue, for those that show little tolerance may be forced to feed on non-homopteran food at this period of the year. Unfortunately, few studies allow an assessment of the relative tolerances of different coccinellids to ants. Most studies of the effects of ants on coccinellids have involved a single target species of ladybird (e.g. Bradley 1973; Itioke and Inoue 1996), or have lumped all coccinellids into one taxonomic category (e.g. Banks and Macaulay 1967; Bristow 1984). However, two studies suggest that there is considerable variation in the tolerance of coccinellids to ants, even if the myrmecophilous coccinellids are excluded.

DeBach *et al.* (1951) observed the ant *Iridomyrmex humilis* tending *A. aurantii* on *Citrus* affected *Rhyzobius lophanthae* and a *Chilocorus* species to different extents. Thus, while 66% (80 of 122) of the former ladybird were found in the presence of ants, only 15% (33 of 218) of the latter were.

In a study in an English pine forest, the numbers of six species of coccinellid, two types of aphid, and the presence or absence of ants were monitored from spring to autumn (Sloggett 1998; Sloggett and Majerus 2000a, 2000b). The ladybirds comprised four conifer specialists, *Myrrha 18-guttata*, *Anatis ocellata*, *Myzia oblongoguttata* and *Harmonia 4-punctata*, the generalist *C. 7-punctata*, and the myrmecophile *Coccinella magnifica*. The aphids were *Schizolachus pineti*, which is not tended by ants, and *Cinara* species, which is. The site was split into two adjacent sections, one of which contained a number of nests of *F. rufa* (ant plot), while the other was free of *F. rufa* (control plot). *Cinara* aphids on *Pinus sylvestris* in the ant plot were regularly tended by *F. rufa*, while those in the control plot were not.

The patterns of abundance of the various ladybirds in the ant plot and control plot through the summer were analysed to allow deductions on the tolerance of the different species to be made. *Myrrha 18-guttata* and *A. ocellata*, had little tolerance of ants, these species only occurring in the ant area after the ants had disappeared in September. A third species, *H. 4-punctata*, had low ant tolerance, for although it was occasionally found in the ant plot, it was much less abundant here than in the control plot. The same is true of *C. 7-punctata*, although the tolerance of this species to ants seems to be somewhat higher. *Myzia oblongoguttata* was found significantly more in the control plot than in the ant plot in early summer, when aphids were abundant. However, once aphids became scarce, it was found as commonly in the ant foraging area as elsewhere. Importantly, it increased in abundance in the ant area once aphids became scarce, strongly suggesting that it moved into the ant areas to feed on *Cinara* aphids even though these were ant-tended. *Myzia oblongoguttata* is highly specialized in its diet, only breeding when feeding on conifer aphids, particularly *Cinara* spp. (Majerus 1993). This specialization on a limited number of aphid species most of which elicit ant-attendance will have imposed selection pressure for *M. oblongoguttata* to be tolerant to ant attacks. The main defense observed was physical, the ladybird dropping the elytra down to the substrate on the side being assailed by ants. Occasionally *M. oblongoguttata* was observed to run from ants, but it rarely dropped from the pines and was not observed to fly away. The sixth species of ladybird, *C. magnifica* was more abundant in the ant area than in the area lacking ants throughout the study, confirming its myrmecophile status (see below).

Larvae of five species were found on *P. sylvestris*, those of *C. 7-punctata* not being found. No larvae of *M. 18-guttata* were found in the ant area. Larvae of *A. ocellata*, *H. 4-punctata*, and *M. oblongoguttata* were much more abundant in the ant free area than in the ant area. Larvae of *C. magnifica* were confined to the ant area.

On the basis of these results, Sloggett and Majerus (2000a), drew up an order of ant tolerance for these six ladybirds: *M. 18-guttata* + *A. ocellata* < *H. 4-punctata* < *C. 7-punctata* < *M. oblongoguttata* < *C. magnifica*. They concluded that *C. magnifica* is a true myrmecophile, while *M. oblongoguttata* has some defense against ants both as an adult and larva, and appears to use ant-tended aphids, particularly in late summer when *Cinara* aphids are almost all ant-tended. Adult *C. 7-punctata* are able to coexist with *F. rufa* at moderate levels when aphids are scarce, but does not breed in the presence of *F. rufa*.

Coccinellid defenses against ants

The tolerance of different species of ladybirds to ants will depend at least in part on the defensive capabilities of the ladybirds. A variety of mechanisms are used by coccinellids when faced with ant aggression (Pasteels *et al* 1973; Richards 1980, 1985; Majerus 1994). These defenses may be behavioural, physical or chemical, with some being shown at specific periods of the life cycle, while adults and immature stages share others.

Behavioural defenses

When coccinellids are attacked by ants, most show some form of defensive behaviour. Most commonly, larvae seek to escape by running away, or dropping to the ground, while adults can use these tactics or may fly (Banks 1962; Bradley 1973; Itioka and Inoue 1996). For adults an alternative to fleeing is to 'clamp down', retracting their legs under the body, pulling their heads in close to the thorax and attaching themselves firmly to the substrate that they are upon (Bradley 1973; Jiggins *et al.* 1993; Majerus 1994). This behaviour is shown by many coccinellids, particularly those of the sub-family Chilocorinae. Members of this sub-family have a very flat ventral surface and a lip around the edge of their elytra, so that when they clamp, the contact made with a flat substrate is very tight, and prevents ants from gaining access to the less well protected ventral surface of the ladybird. Many species of the sub-family Coccinellinae do not clamp down completely, but adopt a rolling motion, dropping the side being attacked to make close contact with the substrate, and again guarding the ventral surface against ant attack (Jiggins *et al.* 1993; Sloggett 1998).

The oviposition behaviour of many predatory ladybirds may also increase fitness by reducing the loss of eggs to ants. The little evidence on the response of ants to coccinellid eggs is equivocal. Banks (1962) recorded *Lasius niger* removing eggs of *A. bipunctata* from colonies of *Aphis fabae*, while El-Ziady and Kennedy (1956) did not observe this behaviour in tests with the same species of ant and ladybird. Many aphidophagous ladybirds lay batches of eggs some short distance from aphid colonies, which may reduce interactions with tending ants. However, this behaviour is more likely to have evolved to reduce predation of eggs by predators such as syrphid, neuropteran and coccinellid larvae, including conspecifics, that are foraging on aphid colonies (Majerus and Majerus 1997a; Sloggett 1998). Most coccidophagous ladybirds lay eggs singly or in batches of two or three in crevices in the substrate, or under dead or live coccids or coccid exuviae (Clausen 1940; Ahmed and Ghani 1966; Drea and Gordon 1990; Majerus 1994). Again avoidance of egg predation by coccidophagous predators was probably a more important evolutionary pressure than ant attack in the development of such behaviour. Moreover, oviposition under live coccids provides neonate larvae with easy access to the more vulnerable ventral surface of a prey whose hard dorsal surface may prevent predation (Drea 1978).

Many coccinellid pre-pupae and pupae, attached to the substrate by their anal cremaster, can rapidly raise their anterior end in response to tactile stimuli. This 'pupal flicking' behaviour (Majerus 1994) may be repeated many times. It has been suggested that this behaviour is a defense against ants, with the joints between abdominal segments of the pupae acting as 'gin-traps' that damage ant appendages (Attygalle *et al.* 1993a). However, a more likely explanation of this behaviour is that it acts to reduce oviposition by pupal parasitoids, such as scuttle flies (Diptera: Phoridae) (Majerus and Kearns 1989; Disney *et al.* 1994; Majerus 1994)

It is unclear whether these behavioural defenses evolved as specific responses to ant aggression, or are general anti-predator or anti-parasitoid devices, although Sloggett (1998) suggests that some of these behaviours are more extensively developed in species that encounter ants frequently.

Physical defenses

The chorion of ladybird eggs is relatively thin (Crowson 1981), and is unlikely to be an effective defense against ant mandibles.

The exoskeleton of coccinellid larvae is soft and easy to pierce. However, many coccinellid larvae are covered by spines (Richards 1980), which may provide some protection against ant attack, although this has not been demonstrated experimentally (Sloggett 1998).

The exoskeletons of coccinellid pupae are relatively hard, and although not impregnable to predators or parasitoids (Majerus 1994), it will provide some protection against ant attack. Moreover, except in the Coccinellinae, Sticholotinae and a few species from other sub-families, the pupa gains some protection from the final larval skin (Richards 1980), which is not shed back, but simply splits along the dorsal mid-line during pupation. This additional layer, which is sometimes spiny or waxy, should be considered a general defensive adaptation.

Some coccinellid larvae are covered on the ventral surface by a network of wax filaments. Pope (1979) proposed that this wax covering was an adaptation against ant attack. The wax covering may be adaptive in interactions in several ways. First, it may be difficult for ants to bite into. Second, as some of the waxes are sticky, it may cause ants to break off attacks to clean their mouthparts. The defensive efficiencies of wax coverings of two species of *Scymnus* were examined experimentally by Völkl and Vohland (1996). They showed that mortality of normal larvae (waxy) of *S. nigrinus* and *S. interruptus*, caused by attacks from *F. polyctena* and *L. niger* respectively, was lower than that of larvae from which the wax covering had been removed. Although some normal larvae were still killed, the ants that attacked these larvae frequently broke off attacks to clean wax off their mouthparts. Moreover, Völkl and Vohland (1996) found that numbers of *S. nigrinus* larvae were significantly higher close to *F. polyctena* than in its absence, while numbers of *S. interruptus* were similar in the presence and absence of *L. niger*.

A third adaptive function of the waxy covering of some coccinellid larvae is that it may be a form of mimicry. Some of the species with wax coverings feed on mealy aphids, and resemble their prey closely. It is thus possible that mealy bug tending ants may ignore such coccinellid larvae because they do not recognise the larvae as a threat to the homopterans that they are tending. This possibility is supported by observations of *C. montrouzieri* larvae being ignored by *P. megacephala* when on colonies of waxy mealy bugs tended by this ant, and attacked by the same ant when on tended colonies of the waxless *Coccus viridis* (Bach 1991). The wax coverings of coccinellids are secreted by the larvae themselves. Interestingly, in some chrysopids (Neuroptera), larvae harvest wax from their homopteran prey and stick it to their dorsal surface (Eisner *et al.* 1978). These larvae frequently feed on ant-tended aphids. As the wax coverings of the aphids and chrysopid larvae are indistinguishable, the ants do not attack the larvae.

Some coccinellid pupae also have wax coverings. The larva of *Scymnodes lividigaster* smears wax onto the surface of the substrate where it attaches before pupation (Richards 1980). The pupa that is formed is both waxy-covered and spiny. Richards (1980) has proposed that both the wax smear and the pupal covering act to deter aphid-tending ants, as well as other potential predators, such as syrphid and neuropteran larvae. If so, it is not clear whether any deterrent effect is due to the physical barrier posed by the pupal covering, the texture of the wax, its chemical composition, its colour, or a combination of these (Richards 1980; Sloggett 1998).

The pupa of *Rodatus major* has a very dense wax covering. Again it has been suggested that in addition to being a physical defensive barrier against ant aggression, the covering may have a mimetic function. The species feeds almost exclusively on eggs of the coccid *Monophlebus pilosior*, and Richards (1985) has proposed that the wax covering gives *R. major* larvae a mimetic resemblance to the ovisac of this coccid. As *M. pilosior* is frequently tended by *Iridomyrmex* ants, the mimicry may have evolved as a defense against ant aggression.

Not all waxy coverings of coccinellid larvae act as a protection against ants. There is considerable variation in both the nature and amount of wax produced by different species of ladybird. Thus, for example, the waxy larvae of *C. montrouzieri* are frequently attacked and killed by ants (Bach 1991), while larvae of *Azya* species are often attacked by ants (Cochereau 1969) and are excluded from ant tended prey colonies (Jutsum *et al.* 1981; Reimer *et al.* 1993). The larvae of some coccinellids, such as those of the genus *Rhizobius*, produce very little wax, and Pope (1979) argues that the amount is so small that it will have no deterrent effect against ant attack.

The main physical defense of adult coccinellids against ant attack is its hard dorsal surface, comprising the pronotum and elytra. Coupled with the clamping and rolling behaviours previously mentioned, the dorsal surface provides a stout barrier to injury from ants. It is possible that the fine hairs that cover the elytra of some coccinellids provide additional protection against ant attack, but this has yet to be demonstrated.

Chemical defenses

Coccinellids are well known for their bright colour patterns, which are generally considered to be aposematic, advertising unpalatability (Brakefield 1985; Majerus 1994). This unpalatability is largely chemical in nature. Coccinellids reflex bleed, which is to say they secrete a foul-smelling, distasteful fluid from the tibio-femoral joints of adults or the dorsal surface of larvae and pupae.

At the centre of coccinellid chemical defense lie alkaloids (Daloze *et al.* 1995), pyrazines (Moore *et al.* 1990) and histamines (Frazer and Rothschild 1960). The first alkaloid to be identified was N-oxide coccinelline, and its corresponding free base precoccinelline, extracted from *Coccinella 7-punctata* (Tursch *et al.* 1971). These also

occur in many other species of the genus *Coccinella*, and some other genera (Henson *et al.* 1975). Other alkaloids have been detected in other species. For example, several predatory ladybirds, including *Harmonia axyridis*, produce an aliphatic diamine, harmonine (Braconnier *et al.* 1985, Enders and Bartzen 1991), while several alkaloids have been extracted from the plant-eating ladybird *Epilachna varivestis* (Attygalle *et al.* 1993; Proksch *et al.* 1993). Although many coccinellids synthesize more than one alkaloid, in a particular species or genus one alkaloid usually predominates. Thus in species of *Chilocorus*, chilocorine A or chilocorine B are most commonly present in quantity, while in *Exochomus 4-pustulatus*, exochomine predominates. Among the pyrazines produced by coccinellids is 2-isopropyl-3-methoxy-pyrazine, which is produced by many chemically defended and brightly coloured insects, and is at least partly responsible for the strong smell given off by many coccinellids when disturbed (Rowe and Guildford 1996; Al Abassi *et al.* 1998).

The diversity of alkaloids and other defensive chemicals in the coccinellids, and the variation in concentrations of the various substances present in these cocktails, indicate that these insects were probably some of the first to use what has been termed combinatorial chemistry in their defense (Schröder *et al.* 1998).

Many, but not all of the defensive chemicals found in ladybirds, are synthesized by the ladybirds that bear them (e.g. Tursch *et al.* 1976; Ayr and Browne 1977; Jones and Blum 1983). However, some coccinellids also have the ability to store and use defensive chemicals from their prey. *Coccinella undecimpunctata* and *Adonia variegata* both sequester cardiac glycosides from *Aphis nerii* (Rothschild *et al.* 1973; Rothschild and Reichstein 1976), *C. septempunctata* sequesters pyrrolizidine alkaloids when feeding on *Aphis jacobaeae* (Witte *et al.* 1990). *Hyperaspis trifurcata* gains a major weapon in its armoury by storing anthraquinone carminic acid from its main prey, the cochineal insects of the genus *Dactylopius*. Here the defensive chemicals are manufactured by the plants on which the aphids feed, so the ladybirds get this element of their defense third hand.

The defensive chemicals of many coccinellids have been shown to be distasteful or toxic to many vertebrate and invertebrate predators (Morgan 1896; Pasteels *et al.* 1973; Brakefield 1985; Marples *et al.* 1989), but not to all (Muggleton 1978; Majerus 1994, 1998; Majerus and Majerus 1997b). The general consensus is that reflex blood is distasteful to ants (Stäger 1929; Happ and Eisner 1961; Pasteels *et al.* 1973; Sloggett 1998). Furthermore, ants contaminated by reflex blood may have their mobility impaired by it as it dries out (Stäger 1929; Happ and Eisner 1961; Bradley 1973; Bhatkar 1982).

The extent to which adult coccinellids reflex bleed in response to ant attacks varies between species and circumstances. Some observers have reported that ladybirds hardly ever reflex bleed even under sustained attack by ants (e.g. Marples 1993; Jiggins *et al.* 1993), while others have observed ladybirds reflex bleeding readily when attacked (e.g. Banks 1962; Bhatkar 1982). Majerus (1994) argues that reflex bleeding is used by adult coccinellids as a last defense, used only when other defenses, including fleeing, have failed. Reflex bleeding is costly in terms of energy expended in the synthesis of the chemicals and in fluid loss (de Jong *et al.* 1991; Holloway *et al.* 1991, 1993). Thus, this costly defensive resource will be only be deployed when other strategies have failed, or when the ladybird is in severe jeopardy (Majerus 1994).

Sloggett (1998) has used the cost-benefit argument relating to the reluctance of adult coccinellids to reflex bleed to argue that reflex bleeding did not evolve initially as a defense against ants. He points out that predatory coccinellids most often come into conflict with homopteran tending ants at times of ant-untended homopteran scarcity. At such times ladybirds are likely to have low resource reserves and so reflex bleeding would incur a relatively high cost. In this context it is noteworthy that the phytophagous coccinellid *E. varivestis* reflex bleeds readily when attacked by ants (Happ and Eisner 1961). This species synthesises a vast array of defensive alkaloids (Eisner *et al.* 1986; Attygalle *et al.* 1993a, 1993b; Proksch *et al.* 1993; Shi *et al.* 1996, 1997; Radford *et al.* 1997). Sloggett argues that the complexity of the chemical defenses of *E. varivestis* may be a consequence of its plant diet, which means that, unlike homopteran predators, it will rarely be food limited and so can devote more resources to chemical defense and reflex bleeding.

The evidence relating to coccinellid larvae reflex bleeding in response to ant attacks is clearer than that relating to adults. In short, ladybird larvae readily reflex bleed when attacked by ants (El-Ziady and Kennedy 1956; Happ and Eisner 1961; Bradley 1973; Sloggett 1998). This is probably because, due to their softer exoskeleton, larvae are at greater risk of suffering injury than are adult ladybirds when attacked by ants (Majerus 1994). It is also possibly that as larvae usually occur at times of prey abundance, they are less resource limited than are adults (Sloggett 1998).

Coccinellid eggs and some pupae also have chemical defenses. The eggs of aphidophagous coccinellids are coated in defensive chemicals that deter consumption by some predators, including some non-conspecific coccinellid larvae (Agarwala and Dixon 1992; Majerus 1994; Hemptinne *et al.* 2005), and may also deter ants (Sloggett 1998). For example, *F. rufa* workers find the eggs of *C. septempunctata* repellent, although they may still destroy the eggs (Sloggett 1998). Some coccinellid pupae (e.g. Chilacorini) can reflex bleed and the reflex blood probably has some deterrent effect against ants. Pupae of *E. varivestis* have a covering of hairs. These hairs

are glandular, each hair bearing a droplet of alkaloid at its end. This droplet is repellent to the ant *Leptothorax longispinosus* (Attygalle *et al.* 1993a).

Ant aggression probably played little if any role in the initial evolution of reflex bleeding in coccinellids. However, it may have a role in shaping the precise balance of defensive capabilities of coccinellids to a variety of predators, parasites and pathogens. In species of coccinellid that frequently encounter ants, relatively more resources may be devoted to defenses that are effective against ants (and less to defenses against other enemies) than in species that rarely interact with ants. In addition, ants may reduce the density of potential coccinellid predators and parasitoids occurring within ant forage ranges, producing enemy free space (Jeffries and Lawton 1984). If so, ladybird species that commonly co-occur with ants, including myrmecophilous species, may invest fewer resources in defenses against potential predators and parasites that are excluded by ants, than would ladybirds that rarely occur with ants (Sloggett 1998).

Myrmecophily

The extent of myrmecophily among coccinellids

A small number of coccinellids are regularly associated with one or more species of ant and have been suggested to be myrmecophilous (Berti *et al.* 1983; Sloggett 1998). These are listed in Table 1, together with the ants they are known to be associated with, and the evidence on which their suggested myrmecophily is based. Of the ten taxa listed, the case for myrmecophily is weak and should be considered unproven in five. The suggestion of myrmecophily in species in the tribe Monocorynini is based only on antennal morphology, and records of any association between these ladybirds and ants are lacking (Sloggett 1998). In the cases of *Scymnus fenderi*, *Scymnus formicarius*, and *Thalassa saginata*, the possibility of myrmecophily emanates from small numbers of records of adults or pupae being found with ants. The myrmecophilous status of *Pseudomyrmex ferruginea* is also in doubt as it is based on observations of larvae being found in the hollow spines of Acacia trees that had been abandoned by ants. For all these species, further observations to determine the extent of any association with ants are needed. Experimental work to determine whether these coccinellids gain benefit from any associations found with ants would be even more valuable.

Table 1 Coccinellids that have been suggested as being myrmecophilous. (Adapted from Sloggett 1998.)

Coccinellid	Associated ant(s)	Evidence for myrmecophily	References
Subfam.: Coccidulinae Tribe Monocorynini Various species	Unknown	Adults have compact antennal clubs. Myrmecophily unproven	Kovář 1996
Subfam.: Scymninae <i>Scymnus fenderi</i>	<i>Pogonomyrmex subnitidus</i>	One adult recorded from <i>P. subnitidus</i> nest. Ant is granivorous and does not tend Homoptera. Myrmecophily unproven	MacKay 1983; Hölldobler and Wilson 1990
<i>Scymnus formicarius</i>	<i>Formica rufa</i>	Little known. Adults apparently found with ants	Wasmann 1894
<i>Brachiacantha quadripunctata</i>	<i>Lasius umbratus</i>	Waxy larvae prey upon tended coccids with ant nests. Closely related species are probably also myrmecophilous. Other ant hosts are probable	Mann 1911; Gordon 1985
<i>Hyperaspis reppensis</i>	<i>Tapinoma nigerrimum</i>	Larvae apparently feed on ant-tended fulgorids in ants' nests. Adults are attacked by ants	Silvestri 1903
<i>Hyperaspis acanthicola</i>	<i>Pseudomyrmex ferruginea</i>	Larvae found in hollow spines of <i>Acacia</i> spp. Abandoned by ants. Myrmecophily unproven	Chapin 1966
<i>Thalassa saginata</i>	<i>Hypoclinea bidens</i>	Pupae found with ants. Myrmecophily unproven	Berti <i>et al.</i> 1983
Subfam.: Chilocorinae <i>Platynaspis luteorubra</i>	<i>Lasius niger</i> <i>Myrmica rugulosa</i> <i>Tetramorium caespitum</i>	Multiply recorded with a variety of ant species. Larvae, and pupae show myrmecophilous morphology.	Pontin 1959; Majerus 1994; Völkl 1995
<i>Ortalia pallens</i>	<i>Pheidole punctulata</i>	Myrmecophilous larvae feed on ants. Adult habits unknown	Harris 1921
Subfam.: Coccinellidae <i>Coccinella magnifica</i>	<i>Formica rufa</i> group	All stages found with ants.	Donisthorpe 1919-20; Wasmann 1912; Majerus 1989; Sloggett 1998

The myrmecophily of the remaining five taxa is more certain, but in some of these species rather little is known of the precise nature of the association with ants. In *Brachiacantha quadripunctata*, *Hyperaspis reppensis* and *Ortalia pallens*, myrmecophily may be limited to the larvae. In each species larvae have been found in ant nests feeding respectively on ant-tended coccids, ant-tended fulgorids and the host ants. It is assumed that the predation of ants by *O. pallens* is the result of a dietary shift from homopteran prey after the species had already developed a myrmecophilous habit (Sloggett 1998).

In the remaining two species, *Coccinella magnifica* and *Platynaspis luteorubra* adaptations to a myrmecophilous existence are seen in the larvae and in some other life-history stages. The myrmecophily of both species has been studied in some detail and consideration of these cases sheds light not only on the ecology and evolution of myrmecophily, but on interactions between ants and insects that prey on Homoptera more generally.

The case of Coccinella magnifica

Coccinella magnifica is a known myrmecophile (Morris 1888, Donisthorpe 1919-1920, 1927, Pontin 1959). It occurs through much of the Palaearctic, with a local distribution that results from its association with ants. In north-western Europe, where it has been most closely studied, it is restricted to the foraging areas of the ants of the *Formica rufa* group.

The obligate myrmecophily of *C. magnifica* was confirmed in Britain during a general survey of coccinellids. Because the morphological similarity between *C. magnifica* and *C. septempunctata* could lead to mis-identification, samples of all seven-spotted ladybirds of appropriate size were collected at 26 sites in southern England, without consideration of the presence or absence of *F. rufa*. A total of 5971 ladybirds were collected. Of these, only 49 were *C. magnifica*, all found with *F. rufa*, the remainder being *C. septempunctata* (Majerus 1989).

Donisthorpe (1919-1920) described experiments in which *C. magnifica* and *C. septempunctata* were placed on *F. rufa* nests, the former being only slightly attacked and quickly getting away while the latter was 'vigorously assailed'. Pontin (1959) and Majerus (1989) record similar observations. Indeed, Majerus (1994) notes that in tests in which *C. magnifica* and other species of ladybird were placed on *F. rufa* nests, the other species were vigorously attacked by the ants, while *C. magnifica* was largely ignored, with both larvae and adult *C. magnifica* were treated alike by the ants, sharing in the same immunity. Some workers have recorded that adult *C. magnifica* reflex bleed freely when attacked by ants (e.g. Donisthorpe 1919-1920), while others have observed that its relative immunity to attack by ants was rarely associated with reflex bleeding (Majerus 1989; Jiggins *et al.* 1993).

Various hypotheses have been put forward to explain the low levels of aggression of ants towards *C. magnifica*. Possibly *C. magnifica* secretes some kind of pheromone that placates ants (Majerus, 1989) by advertising distastefulness or toxicity. Alternatively the ladybird may secrete chemicals that mimic the ants' own scent, or possibly the odour of aphids (Majerus 1989). A third hypothesis is that *C. magnifica* exudes a chemical that is harmful to ants (Donisthorpe 1919-1920) or at least acts as a deterrent (Sloggett 1998).

To test the various hypotheses put forward to explain the interactions between *C. magnifica* and ants, Sloggett *et al.* (1998) conducted exhaustive field and laboratory studies of the interactions of *C. magnifica* and other ladybirds that occur with *F. rufa* in conifer and mixed woodland in south-east England. On particular note are experiments investigating the behaviour of *F. rufa* to *C. magnifica* and the closely related *C. septempunctata* introduced onto ant trails and ant-tended aphid colonies. On ant foraging trails *C. magnifica* was attacked occasionally, but very much less than *C. septempunctata*. Moreover, on ant-tended aphid colonies *C. magnifica* remained on the colony longer, and were more successful in feeding on aphids than *C. septempunctata*. Although ants attacked both species, the degree of aggression towards *C. septempunctata* was greater than towards *C. magnifica*. In response to attacks *C. septempunctata* dropped off plants or flew away significantly more often than *C. magnifica*. Both species only rarely responded to ant attacks by reflex bleeding. Finally, none of the defensive behaviours of *C. magnifica* in interactions with *F. rufa* were unique to *C. magnifica*. All were also seen in *C. septempunctata*, with the differences between the species being in the degree to which the various behaviours in the repertoire were used, suggesting that *C. magnifica*'s defense against ants may have evolved by gradual adaptation of *C. septempunctata* behaviours.

Sloggett (1998) also conducted a variety of experiments to investigate the relative defensive chemistries of *C. magnifica* and *C. septempunctata*. He showed that dead *C. septempunctata* were more frequently attacked on ant trails than were *C. magnifica*, and that this was the case when whole corpses, or corpses without elytra or wings, or when just elytra were used. He deduced that the low level of aggression shown by ants to *C. magnifica* is not the result of a defensive behaviour shown by this ladybird, and probably has a chemical basis. Analysis of the cuticular lipids of the two species showed very little difference between them, and little similarity to the surface lipids of *F. rufa*. Due to the lack of similarity between the surface lipids of *C. magnifica* and *F. rufa*, Sloggett (1998) argued that it was unlikely *C. magnifica*'s immunity to ant attacks was due to chemical mimicry of the ants. Moreover, the similarity in the cuticular lipids of *C. magnifica* and *C. septempunctata* made it improbable that *C.*

magnifica gains immunity by mimicking some other element in the habitat. Transfer experiments showed that *C. magnifica*'s defense is not specific to a particular *F. rufa* nest, or indeed to just *F. rufa* (Sloggett 1998). Sloggett concludes that probably *C. magnifica*'s defense against ants is based on repellent chemistry and that the chemicals involved are most probably alkaloids, or possibly pyrazines. Interestingly, while the predominant alkaloids produced by most *Coccinella* species are coccinelline and precoccinelline, that of *C. magnifica* is hippodamine (Dixon 2000). Tests to determine the repellent effects of coccinelline compared to hippodamine on ants would be timely.

Case study: *Platynaspis luteorubra*

Coccinella magnifica is not the only myrmecophilous ladybird. Larvae of *Platynaspis luteorubra* have been found feeding on *Aphis scaliai* in the underground galleries of *L. niger*, and on *Paracletus cimiciformis* in a nest of the turf ant *Tetramorium caespitum* (Pontin 1959; Majerus 1994; Völkl 1995). The larvae and pupae have shapes very unlike those of most ladybirds, but similar to other myrmecophilous larvae, such as some those of lycaenid butterflies and hoverflies of the genus *Microdon*.

Völkl (1995) has shown that *P. luteorubra* is a true myrmecophile and is frequently found in association with underground aphids tended by ants. Intensive field studies on a variety of plants showed that *P. luteorubra* larvae were found significantly more often in ant-tended colonies than in unattended colonies. The species has a range of morphological and behavioural adaptations to life with ants, thereby giving them access to ant-attended resources. Ants do not recognize larvae of *P. luteorubra* as a threat to their attended aphids. This may be a result of the larva's unusual coccid-like shape and its slow inconspicuous movements. Völkl also assumes that the larvae produces 'camouflage' chemicals. More recently, studies in Germany have shown that when larvae of *P. luteorubra* are moved between colonies of two types of ant-tended aphids, *A. fabae* and *Metopeurum fuscoviride*, the response of ants towards them changed. Larvae moved to a new colony of conspecific aphids were not attacked, but those moved to a colony of the other species were (Oczenascheck unpubl. data). Analysis of the cuticular lipids of the larvae showed that these were similar, both in type and quantity, to those of their prey. As the cuticular lipids of the two species of aphid differ both qualitatively and quantitatively, a change in prey led to a change in the cuticular lipids and so the effectiveness of the larvae's chemical mimicry. This is a very efficient form of scent mimicry because the larvae do not have to manufacture different cocktails of mimetic chemicals when feeding on different prey species.

Völkl (1995) found that *P. luteorubra* pupae were frequently attacked by *L. niger*, but were protected from injury by their dense covering of long hairs. It seems then that the chemical mimicry of the larvae is not carried forward into the pupal stage. This is probably because alcohols rather than lipids dominate the cuticular compounds of pupae. Adults were also attacked by *L. niger* and responded either by fleeing or by clamping down on to the substrate.

The myrmecophily of *P. luteorubra* is adaptive. Larvae in ant-tended aphid colonies are more successful in capturing prey than those in unattended colonies, and adults that develop in ant-attended colonies are larger than those that develop in unattended colonies (Völkl 1995).

Habitat specialisation and the evolution of myrmecophily in coccinellids

There are striking differences in the adaptations that the two best-studied myrmecophilous coccinellids have evolved to enable them to live with ants. *Platynaspis luteorubra* larvae chemically mimic aphids, in effect sequestering mimetic chemicals from their prey. *Coccinella magnifica* appears to use ant-repellent chemicals, probably alkaloids, and physical and behavioural defenses. Despite these different adaptations that enable these species to live with ants, the main reason that they do so are probably the same: to enable them to feed on ant-tended aphids when other aphids are scarce. Comparative work on *C. magnifica* and *C. septempunctata* allows speculation on the evolution of myrmecophily. Sloggett (1998) showed that *C. septempunctata* has some tolerance of *F. rufa* during periods of aphid scarcity. Donisthorpe (1919-1920) also wrote of *C. 7-punctata* "experimenting in a myrmecophilous existence". Furthermore, Bhatkar (1982) observed large groups of this ladybird in the vicinity of *Formica polycetena*, while various workers have reported other *Coccinella* species (*C. undecimpunctata*, *C. transversoguttata*, *C. trifasciata*) with ants, particularly in late summer (Bradley and Hinks 1968, Bhatkar 1982). This suggests that members of the genus *Coccinella* often facultatively coexist with ants. Members of this genus are not tolerant of low aphid densities, and this may have driven them to become partially tolerant to ants when aphids are scarce (Sloggett and Majerus 2000a). It thus seems feasible that the non-myrmecophilous ancestors of *C. magnifica* may have occasionally had to prey upon ant-tended aphids, and thus selection was imposed on these ancestors to evolve some degree of tolerance to ants. Additional selective advantages to myrmecophily may have enhanced the behaviour over time. These may have included more efficient use of particular prey species that are frequently ant-tended, reduced energetic costs associated with prey switching (Hatingh and Samways 1992),

reduced requirement for hazardous migrations, reduced competition with other aphid predators and reduced densities of ladybird predators and possibly parasitoids (Sloggett and Majerus 2000a).

Living in the forage range of aggressive ants may confer on myrmecophilous ladybirds an advantage from 'enemy-free space'. Little work has been conducted on the role of enemy-free space in the evolution of habitat or host plant preferences in the coccinellids. However, both *P. luteorubra* and *C. magnifica* provide strands of circumstantial evidence suggesting a role for enemy-free space in the evolution of habitat preferences in coccinellids, although it is unlikely that this role is as widely important as that of prey availability (Sloggett and Majerus 2000a). In *P. luteorubra*, Völkl (1995) found evidence that by living with ants, the levels of infection by the host specific parasitoid wasp *Homalotylus platynaspidis* were reduced. Similarly, Majerus (1994) reported that infestation levels of *C. magnifica* by the wasp *Dinocampus coccinellae*, in conifer woodland in the presence of *Formica* ants, were less than a fifth of those reported from *C. septempunctata* in ant free conifer habitats close by. However, in this case, Sloggett (1998) has argued that the low prevalence of *D. coccinellae* in *C. magnifica* is a consequence of the extremely repellent chemistry of this ladybird rather than a deterrent effect of the ants this ladybird lives with.

In research on habitat or host plant preferences, two questions should be addressed. First, and obviously, one needs to ask why does a species live in certain habitats or on certain plants? The second question, which is often forgotten, is why does a species not live in other habitats or on other plants? In other words, we need to ask what makes a particular habitat favourable while others are unfavourable. Species with highly specialized ecologies, such as myrmecophilous species, are easiest to interpret.

In the case of *C. magnifica*, we have some idea of why it lives with ants and not elsewhere. They do so to utilize a food source protected from other predators by an aggressive guardian, against whom they are themselves well defended. They may also be protected from their own enemies by these aggressive predators. But why does *C. magnifica* not live anywhere else? Here we must speculate. Possibly *C. magnifica* is a poor competitor or lacks efficient defenses against predators and parasites. But, if this is so, more fundamental questions must then be asked: why are they bad competitors or their defenses inadequate?

The reason that *C. magnifica* is either a bad competitor, or has inefficient defenses, may follow from its specialization to life with ants. Production of repellent alkaloids has an energetic cost, reducing resources available for other functions, such as production of toxins, or fighting ability. This is a direct cost of immunity. A more indirect cost may be that when living in the proximity of *Formica* nests, the selection pressures that might promote the evolution and maintenance of strong defenses against a range of predators and parasites are reduced, because the ants keep these enemies at bay. If any cost is incurred in having these ancestral defense systems, the systems are likely to be lost. If encounters with potential predators or parasites become rare events, selective disadvantages, incurred by their costs, will outweigh the selective advantages from their maintenance.

In the early 1990s, a colleague, Dr John Barrett, devised an interesting analogy. If *C. magnifica* are the populace of the United States, then the ants could be seen as a Reaganesque Star Wars system, spreading a powerful defensive umbrella over an area surrounding their territory. Presence of this defensive system negates the need for more old fashioned, conventional defenses, and the costs of these can be saved. One is safe as long as one stays under the umbrella, but not if one strays.

Conclusion

Many predatory coccinellids encounter homopteran-tending ants regularly. In consequence, ants are an important factor in the ecology of many coccinellids. However, caution should be taken when investigating the interactions between coccinellids and ants. Perhaps too often, the reactions of coccinellids to ants have been viewed in isolation. In reality, many of the defenses used by coccinellids when faced with aggressive ants are simply slight adaptive modifications of general defenses. Consequently, it is those few species of coccinellid that have the closest association with ants, the myrmecophilous ladybirds that may be most illuminating. In these species, the closeness of the association means that many of the behaviour of the coccinellids to ants have evolved specifically because of the association. The two best studied myrmecophilous coccinellids, *C. magnifica* and *P. luteorubra*, have already given insights into not only specific inter-species interactions, but also the roles of enemy free space, resource utilization, inter-specific competition and the evolution of habitat specificity. Moreover, the case studies of these two species show both illuminating similarities and differences. It is unlikely that all myrmecophilous coccinellid have been identified. If other ladybirds with close associations with ants are identified, and then closely scrutinized, they will surely provide novel insights into a range of phenomena.

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