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Adaptations of *Coccinella magnifica* Redtenbacher, a Myrmecophilous Coccinellid, to Aggression by Wood Ants (*Formica rufa* Group). I. Adult Behavioral Adaptation, Its Ecological Context and Evolution

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Coccinella magnifica *is an obligate associate of* Formica rufa-*group ants. The congener* Coccinella septempunctata *can serve as a model for its nonmyrmecophilous ancestor.* Formica rufa *behavior toward these two ladybirds, and their behavior, was compared. Although* C. magnifica *was rarely attacked on ant trails, it was usually attacked on tended aphid colonies.* Coccinella septempunctata *was more readily attacked. The two ladybirds' behavior was similar on trails, but* C. magnifica *used more defense and* C. septempunctata *more rapid escape behavior on aphid colonies. Only* C. magnifica *fed upon tended aphids. Chemical adaptation to overcome ant aggression probably exists in* C. magnifica, *but it possesses almost no novel behaviors to counter ant aggression. Instead, modifications have occurred in the expression of behaviors present in* C. septempunctata.

KEY WORDS: *Coccinella magnifica*; *Coccinella septempunctata*; *Formica rufa*; ant-tended aphids; ant aggression; myrmecophily.

INTRODUCTION

The interaction between ants and homopteran-eating insects is an adversarial one. Ants prey upon such homopteran-eaters as part of an insect diet (Way, 1963;

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Chauvin, 1966; Mariau and Julia, 1977). More importantly, ants tend colonies of many Homoptera in order to obtain honeydew, a rich source of carbohydrate, which they frequently defend, attacking predators and parasites seeking access to these colonies (Way, 1963; Buckley, 1987; Hölldobler and Wilson, 1990).

Although the degree of deterrence resulting from ant attendance is variable, many homopteran-eaters are less frequent visitors to well tended colonies of Homoptera (e.g., Bartlett, 1961; Banks and Macaulay, 1967; Bradley, 1973; Nakashima *et al.*, 1976). However, a number of species are recognized as regular, even obligate, associates of ants, feeding upon the tended homopteran resource with apparent impunity (e.g., Pontin, 1959). Like all myrmecophiles, they display a variety of adaptations assisting in the avoidance of ant aggression. These include cautious movement or avoidance behavior (Völkl, 1995, 1997), "limuloid" (flattened) body shape (Pontin, 1959; Völkl, 1995), physical camouflage or protection (Eisner *et al.*, 1978), and chemical mimicry (Takada and Hashimoto, 1985; Liepert and Dettner, 1996). One aphid hyperparasitoid utilises chemical defense and repellence in order to overcome ant aggression (Völkl *et al.*, 1994).

The aphidophagous coccinellid (ladybird beetle) *Coccinella magnifica* Redtenbacher (=*C. distincta* Faldermann, *C. labilis* Mulsant, *C. divaricata* Olivier, *C. lama* Kapur) is usually considered to be an obligate associate of wood ants, *Formica rufa* group (Donisthorpe, 1896, 1919–1920; Wiśniewski, 1963; Majerus, 1989; Sloggett, 1998; but see also Wasmann, 1912; Pontin, 1959). All life history stages are found in the presence of the ant where they are believed to feed upon tended aphids, although previously there have been remarkably few observations of *C. magnifica* feeding in the wild (for observations of larvae feeding see Donisthorpe, 1919–1920; Pontin, 1959). *Coccinella magnifica* are often found walking on the ants' foraging trails (Morris, 1888; Champion, 1903; Donisthorpe, 1919–1920), where they may perhaps additionally feed upon aphids which have fallen from vegetation.

Previous authors have observed that *C. magnifica* is not attacked by wood ants, although most other ladybirds are (Donisthorpe, 1919–1920; Majerus, 1989). *Formica rufa* L. workers frequently tap *C. magnifica* with their antennae without displaying obvious signs of aggression (Donisthorpe, 1919–1920; Majerus, 1989). Majerus (1989) found *C. magnifica* adults and larvae never to be attacked by *F. rufa* workers if placed near *F. rufa*-tended aphids, unlike other ladybird species. He considered it probable that *C. magnifica* possessed some form of chemical adaptation to overcome *F. rufa* aggression.

Possible behavioral adaptations have also been noted in *C. magnifica*. Donisthorpe (1900) observed that the ladybird would frequently "duck down," stopping briefly and drawing its legs and antennae close to its body, when it encountered an *F. rufa* worker. Donisthorpe (1903) also described *C. magnifica* chemical defense in response to *F. rufa* attack (see below). Arnold *et al.* (cited by Majerus, 1994) observed that *C. magnifica* adults and larvae ignored *Lasius niger*

L. worker attacks when placed on colonies of aphids that this ant was tending. This led Majerus (1994) to suggest that *C. magnifica* had lost a typical ladybird "fleeing response" due to its association with the *F. rufa* group. It should be noted that *C. magnifica* does not associate with *L. niger* on a regular basis (Sloggett, 1998).

Coccinella magnifica is the only member of the ladybird subfamily Coccinellinae which is known to indulge in myrmecophily. Furthermore, its genus is both widespread and speciose (Iablokoff-Khnzorian, 1982; Gordon, 1985). As a phylogenetically isolated myrmecophile, with extant close relatives, it is a highly suitable subject in which to study of the nature and evolution of its adaptations to overcome ant aggression.

Coccinella magnifica bears a striking resemblance to the nonmyrmecophilous *Coccinella septempunctata* L., to which it is closely related (Donisthorpe, 1919–1920; Majerus, 1989). The two species are placed in the same subgenus, on morphological grounds (Iablokoff-Khnzorian, 1982), although it is unknown whether they are sibling species. Apart from similarities of size and color pattern (Dobzhansky and Sivertzew-Dobzhansky, 1927; Dobzhansky, 1933; Filippov, 1961; Iablokoff-Khnzorian, 1982), the two species share a number of aspects of their ecology. They occur on a wide variety of host plants (Majerus, 1991, 1994) and they can both feed upon a similarly broad range of aphid species (Sloggett, 1998; J. J. Sloggett, unpublished data). The overwintering biology of the two species is similar and they may even overwinter together in mixed groups, during a period when *F. rufa*-group ants are also overwintering (Majerus, 1992, 1994). Both species have large natural Palaearctic ranges, which overlap in great part (Iablokoff-Khnzorian, 1982) and exhibit similar voltinism (Majerus, 1994). The two species' close relatedness and the large number of shared biological characters, with many probably arising by common descent, make *C. septempunctata* an ideal model species for the nonmyrmecophilous ancestor of *C. magnifica*.

By comparing *C. magnifica* to *C. septempunctata*, the adaptations of *C. magnifica* to coexistence with *F. rufa*-group ants may be elucidated and their evolution from the *C. septempunctata* state considered. Specific adaptations of *C. magnifica* to overcome ant aggression may be separated from more general antipredator defenses found in *C. septempunctata*. For example, reflex bleeding, whereby distasteful, repellent fluid is released from the tibiofemoral joints of ladybird adults, is used by *C. septempunctata* and many other species to repel a wide variety of invertebrates and vertebrates, including ants (Pasteels *et al.*, 1973; Bhatkar, 1982; Brakefield, 1985; Majerus, 1994). *Coccinella magnifica* may chemically defend itself by reflex bleeding (Donisthorpe, 1903), but this could not be described as a specific adaptation of this ladybird to ants.

In this paper the behavioral interactions between adult *C. magnifica* and one wood ant species, *F. rufa*, are considered. The likelihoods of *F. rufa* attack on

C. magnifica and *C. septempunctata* adults are compared under two ecological contexts, on *F. rufa* foraging trails and on colonies of *F. rufa*-tended aphids. The behavior of *F. rufa* and the two ladybird species during encounters on trails and colonies of tended aphids is described. In the discussion, the role of ecological context in dictating the relative importance of behavioral adaptation and the evolution of *C. magnifica* behavior from the *C. septempunctata* state are considered.

MATERIALS AND METHODS

Field Site and General Considerations

The experiments described here were carried out at Esher Common and Oxshott Heath, Surrey, England. Both study areas, which are separated by a distance of approximately 1 m, consist of mixed *Erica* and *Calluna* heathland, with Scots pine, *Pinus sylvestris* L., birch, *Betula* sp., oak, *Quercus* sp., and sweet chestnut, *Castanea sativa* Miller. *Formica rufa* nests are common in parts of both areas. *Coccinella magnifica* may be found on trees and low-growing vegetation, where *F. rufa* is present, and *C. magnifica* adults and larvae are also observed walking on *F. rufa* foraging trails. *Coccinella septempunctata* also occurs at the study sites, primarily where *F. rufa* is absent, although specimens are sometimes found in the vicinity of *F. rufa* colonies (Sloggett, 1998).

These experiments were carried out between 1995 and 1997, mainly in late summer and early autumn (July to October). At this time of year ants were tending diminishing numbers of aphids in well-formed colonies. The *F. rufa*-tended aphid colonies used in this work were all on *P. sylvestris*. The aphid species was identified as *Cinara pini* (L.) (C. I. Carter, personal communication).

Wild adult *C. magnifica* and *C. septempunctata*, collected by beating vegetation or by eye, were used in all experiments, with *C. magnifica* always used in the same area in which it had been collected. All experiments were carried out as matched trials, to control for changes in *F. rufa* aggression related to temperature, time of day, or season. Within a trial, individual *C. magnifica* and *C. septempunctata* were introduced sequentially onto the same ant trail or aphid colony. Order of introduction was randomized between trials in the first experiment or reversed each time in subsequent experiments. Within trials on tended aphids a gap of at least 15 min was left between ladybirds placed on the same colony. Many of the data sets collected are in the form of frequencies, and in these cases less powerful unpaired tests (χ^2 tests and Fisher exact tests) were used in analysis.

Formica rufa Aggression Toward *C. magnifica* and *C. septempunctata* in the Presence and Absence of Tended Aphids

The likelihood of attack by *F. rufa* on *C. magnifica* and *C. septempunctata* was compared in two ecological contexts: on *F. rufa* foraging trails and on colonies of *F. rufa*-tended aphids. Adult ladybirds were released individually, either onto a *F. rufa* foraging trail, on the ground, or near a colony of *F. rufa*-tended *C. pini*. Within matched trials ants on trails and tending aphids originated from the same nest.

The number of *F. rufa* workers encountering each ladybird until it was attacked was recorded. Thus, a score of 1 indicated that the first ant encountering a ladybird attacked it. A score of 10 would indicate that the first 9 encounters between *F. rufa* and the ladybird did not result in the ladybird being attacked but the 10th encounter did. If the ladybird was not attacked after 10 encounters, it was recorded as >10 or unattacked. A ladybird was considered attacked if an *F. rufa* worker attempted to bite the ladybird or spray it with formic acid by pulling its abdomen under the rest of its body. As the number of ants tending aphid colonies on individual branches was often small, multiple encounters between individual ants and ladybirds were counted separately. Ladybirds leaving the *F. rufa* trail or vicinity of tended aphids before 10 encounters were replaced on the trail or colony. Each ladybird was tested only once, on either an ant trail or an aphid colony.

Behavior of Ants and Ladybirds on *F. rufa* Foraging Trails

Adults of the two ladybird species were placed upon *F. rufa* foraging trails. The behavior of *F. rufa* workers and ladybirds were recorded for the first five encounters observed between ants and an individual ladybird. Ladybirds which walked off the trail before five encounters were observed were replaced on the trail.

Ant behavior toward ladybirds was assigned to one of four categories: no visible response to the ladybird, orientating toward the ladybird, tapping the ladybird with its antennae, and attacking the ladybird. If a second ant joined an attack on a ladybird, this was not recorded as a separate encounter, since the behavior of the first ant might have influenced that of the second.

Observations of ladybird behavior concentrated primarily upon ladybird movement. This included whether the ladybird was moving or stationary before an encounter and whether it ran, stopped, or stayed still during an encounter. Defensive behaviors, such as reflex bleeding, were also recorded.

Behavior of Ants and Ladybirds on *F. rufa*-Tended Aphid Colonies

Individuals of *C. magnifica* and *C. septempunctata* which had been starved overnight were placed near individual *F. rufa*-tended colonies of *C. pini* and allowed to walk onto the colony. Each individual was recorded as attacked on unattacked by *F. rufa* while on the aphid colony. Ladybirds were recorded as unattacked only if none of the ants they encountered while on the colony attacked them. Very rare individuals which left the colony without encountering ants were returned to the colony. Defensive behavior of ladybirds on aphid colonies was recorded, as was the ladybird method of escape (running or dropping) from the colony ($n = 16$ paired trials). In some of these trials the times which individuals of each species remained on aphid colonies were also recorded ($n = 6$) and extra trials were carried out, where ladybird behavior was not recorded, but time on the colony was ($n = 11$). Ant attacks were recorded, as was any feeding on the part of the ladybird in all cases (total $n = 33$ paired trials). Some aphid colonies were used more than once, when accessible *C. pini* colonies were difficult to find.

RESULTS, ANALYSIS, AND CONCLUSIONS

Formica rufa Aggression Toward *C. magnifica* and *C. septempunctata* in the Presence and Absence of Tended Aphids

Formica rufa workers attacked *C. magnifica* adults on *F. rufa* foraging trails less readily than they attacked *C. septempunctata* adults in the same location (Fig. 1a). The reduction in *F. rufa* aggression toward *C. magnifica* was highly significant (two-tailed Wilcoxon signed ranks test, counting a score of >10 as 11: $n = 14$ untied observations + 4 ties, $T^+ = 104$, $P = 0.0002$).

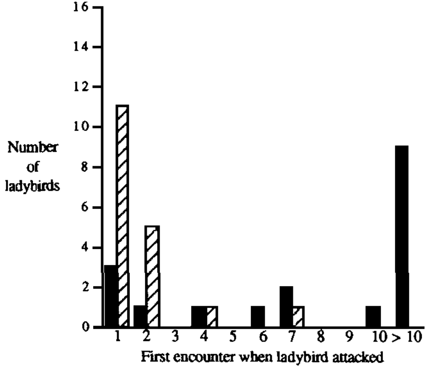
When tending aphids, the frequency of aggression displayed by *F. rufa* workers toward the two species was more similar (Fig. 1b). The frequency of *F. rufa* aggression toward adult *C. magnifica* was significantly less than that for *C. septempunctata* (two-tailed Wilcoxon test: $n = 7$ nonties + 11 ties, $T^+ = 28$, $P = 0.015$), although it must be noted that the Wilcoxon test does not take into account a large number of tied observations. The single observation of an unattacked *C. magnifica* in these data (Fig. 1b) was made during cold, wet weather when the attendant ants are less active (see Way, 1963).

That *F. rufa* attacked *C. magnifica* more readily when tending aphids is supported by the high significance of a comparison of the reaction of ants toward *C. magnifica* adults on foraging trails and colonies of tended aphids (two-tailed Wilcoxon test: $n = 14$ nonties + 4 ties, $T^+ = 105$, $P = 0.0002$). It appears that *F. rufa* workers are generally more aggressive toward ladybirds when tending aphids. This suggested by the significance of the same comparison for *C. septempunctata* (two-tailed Wilcoxon test: $n = 6$ nonties + 12 ties, $T^+ = 21$, $P = 0.03$), although a large number of tied values also occur in this data.

Behavior of Ants and Ladybirds on *F. rufa* Foraging Trails

In analyzing the data presented here, each encounter between ant and ladybird has been assumed to be independent. However, the use of each ladybird to obtain data for five ant–ladybird interactions means that this analysis must be treated with some caution.

(a) *Formica rufa* foraging trails



(b) *Formica rufa*-tended aphid colonies

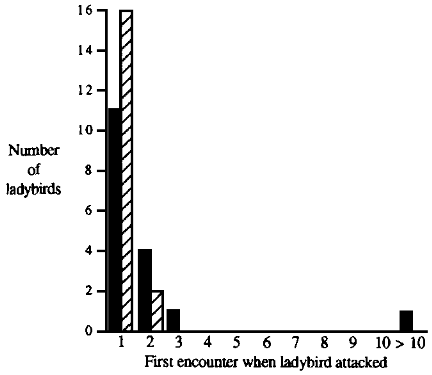


Fig. 1. Frequency distribution of *F. rufa* attacks on *C. magnifica* (black bars) and *C. septempunctata* (striped bars) (a) on ant foraging trails, on the ground, and (b) on tended colonies of aphids.

Formica rufa *Behavior*

The four kinds of *F. rufa* behavior toward ladybirds can be considered to represent a sequence of escalating ant reaction, starting from no response, through orientation, antennation, to finally attacking the ladybird. Some ants stop at each stage; others continuing further in the sequence. In comparing each behavior toward *C. magnifica* and *C. septempunctata*, the frequency of *F. rufa* individuals stopping at a particular stage was compared to that of ants advancing further in the sequence. Thus data from ants stopping at particular behaviors were not included in analyses of later behaviours in the sequence. A series of 2 × 2 partitions were used to compare the frequencies of ants ending the interaction on particular behaviors for the two ladybird species.

This analysis suggested considerable differences in ant behavior toward *C. magnifica* and *C. septempunctata* (Table I). *Formica rufa* workers showed no visible response to *C. magnifica* significantly more often than to *C. septempunctata*. Of those interactions where an *F. rufa* worker visibly responded, interactions ending in orientation occurred at a similar frequency for both species. The ladybirds involved in interactions ending in orientation were virtually all moving rapidly past an ant (nine of nine *C. magnifica*, seven of nine *C. septempunctata*), probably preventing the ant reaction from escalating to the next stage. At this next stage, where *F. rufa* reacted to the continued presence of a ladybird, *C. magnifica* was often only antennated, and ant behavior described by Donisthorpe (1919–1920) and Majerus (1989). *Coccinella septempunctata* was usually attacked, the difference in the frequency of these two ant behaviors toward

Table I. *Formica rufa* Behavior Toward *C. magnifica* and *C. septempunctata* Adults on Foraging Trails[a]

	Number of interactions		χ^2 test	
	Coccinella magnifica	*Coccinella septempunctata*	X^2	P
Ant behavior toward coccinellids: $n = 70$ for both coccinellid species[b]				
Interaction ends with				
No visible response	21	9	6.11	<0.02
Orientation	9	9	0.26	>0.50
Antennation	22	5	22.08	<0.001
Attack	18	47		
Heterogeneity X^2			28.44 (3 df)	<0.001
Effect of coccinellid movement on likelihood of ant attack				
Ladybirds attacked when moving (total moving)	14 (60)	47 (65)		
Ladybirds attacked when stationary (total stationary)	4 (20)	2 (8)		
X^2	0	5.24		
P	1.0	<0.05		

[a] All χ^2 tests have 1 df unless stated otherwise. Those with 1 df, except partition χ^2, were calculated using Yates' correction for continuity.

[b] Two paired data sets are excluded, as five interactions were not recorded for *C. septempunctata*, in order to equalize the number of ant encounters per ladybird for both species.

the two ladybirds being highly significant. In total, 67% of interactions involving *C. septempunctata* ended in an ant attack, but only 26% of interactions involving *C. magnifica* ended in a similar manner.

Ladybird movement appeared to be important in dictating the likelihood of *F. rufa* attack on *C. septempunctata*, with moving ladybirds being attacked significantly more often. This was not so in *C. magnifica.*

Coccinella magnifica *and* C. septempunctata *Behavior*

During the course of observations, 2 *C. septempunctata*, of 16, flew away, before five interactions occurred. Other *C. septempuncata* were recorded exhibiting brief flight during observations. No *C. magnifica* were observed to fly. At least four *C. septempunctata* reflex bled, although no *C. magnifica* were observed to do so. It seems likely that both these differences arise from the increased *F. rufa* aggression displayed toward *C. septempunctata. Coccinella magnifica* was often observed to hide under debris on the ground, as noted by earlier authors (e.g., Donisthorpe, 1896). This may act to reduce direct encounters between *C. magnifica* and *F. rufa* on foraging trails. *Coccinella septempunctata* individuals rarely hid, although this may have been due to the high frequency of ant attack agitating the ladybirds.

The main analysis comparing *C. magnifica* and *C. septempunctata* behavior concentrated upon the importance of movement. Since attacks on *C. septempunctata* more often occurred on moving ladybirds, it was considered possible that the "ducking down" behavior of *C. magnifica* (Donisthorpe, 1900) might be of importance in rendering *F. rufa* less likely to attack *C. magnifica* through cessation of movement.

Recorded encounters between each species and *F. rufa* were divided into four groups on the basis of whether the ladybird was moving or stationary before an encounter and whether or not the ant attacked the ladybird. Individuals in each group were dichotomized on the basis of whether they initially were still or ran in response to encountering or being attacked by an ant. The frequency of reactions of *C. magnifica* and *C. septempunctata* were compared for each group using a χ^2 test or a two-tailed Fisher exact test (Siegel and Castellan, 1988).

This analysis suggested that, given a particular ant reaction (attack or not), the behavior of the two species was very similar (Table II). When stationary and encountered by ants, both *C. magnifica* and *C. septempunctata* usually remained so, whether attacked or unattacked. Moving individuals of both species often stopped, at least briefly, when attacked. Both species also were observed stopping when unattacked, the "ducking down" described by Donisthorpe (1900) for *C. magnifica*, but not observed by him for unattacked *C. septempunctata*. In all four groups, probabilities of congruence between frequency data for the two

Table II. *Coccinella magnifica* and *C. septempunctata* Movement in the Presence of *F. rufa*[a]

	Number of interactions		
	Coccinella magnifica	*Coccinella septempunctata*	χ^2 test/two-tailed Fisher exact test
Ladybird response when attacked by a *F. rufa* worker			
When stationary			
Stay still	3	1	Fisher exact test: $P = 0.60$
Run away	1	1	
When moving			
Stop initially	9	35	$X^2 = 0.17$, 1 df, $P > 0.70$
Continue moving	5	12	
Ladybird response on encountering a *F. rufa* worker but not attacked			
When stationary			
Stay still	15	6	Fisher exact test: $P = 0.73$
Run away	1	0	
When moving			
Stop initially	22	10	$X^2 = 0.08$, 1 df, $P > 0.80$
Continue moving	24	8	

[a] χ^2 tests were computed using Yates' correction for continuity.

species were high ($P > 0.50$), although for attacked stationary ladybirds the data set is extremely small. While this frequency data should be treated with caution (see above), it does appear that the behaviors of the two species on *F. rufa* foraging trails are very similar, in both their nature and their probability of occurrence. Although it appears that the "ducking down" behavior is not important in deterring ant attack on *C. magnifica*, as *C. septempunctata* is also observed behaving in this manner, it may be important for both species in ensuring that their appendages are not damaged if an attack occurs.

Behavior of Ants and Ladybirds on *F. rufa*-Tended Aphid Colonies

Ant and ladybird behavior for *C. magnifica* and *C. septempunctata* on tended aphid colonies is summarized, and results for the ladybird species are compared, in Table III. There was no significant difference in the recorded frequencies of attacks by *F. rufa* on the two ladybird species, with only four

Table III. *Formica rufa* and Ladybird Behavior on Colonies of Tended Aphids[a]

Comparison	*n* for each species	*Coccinella magnifica*	*Coccinella septempunctata*	Statistical test
Ant behavior on colony				
Ladybirds attacked	33	33	29	$X^2 = 2.40$, 1 df, $P > 0.10$
Ladybirds seized	22	1	4	$X^2 = 0.90$, 1 df, $P > 0.30$
Ladybird dragged from colony	22	0	1	$X^2 = 0$, 1 df, $P = 1.0$
Ladybird behavior on colony				
Feeding upon tended aphids	33	6	0	$X^2 = 4.58$, 1 df, $P < 0.05$
Defensive	22	20	8	$X^2 = 11.88$, 1 df, $P < 0.001$
Reflex bleeding	22	0	3	$X^2 = 1.43$, 1 df, $P > 0.20$
Ladybird escape from colony				
Run initially	22[b]	19	9	$X^2 = 7.14$, 1 df, $P < 0.01$
Drop initially		3	12[c]	
Time on colony	16	27 sec	6.5 sec	Wilcoxon signed ranks test for large samples: $T^+ = 123$, $z = 2.84$, $P = 0.0046$

[a] Wilcoxon test is two tailed; χ^2 tests were calculated using Yates' correction for continuity.

[b] $n = 21$ for *C. septempunctata*, as one individual was dragged off the colony by ants.

[c] Data on *C. septempunctata* dropping include four individuals which dropped as soon as they encountered an ant, without an attack occurring.

C. septempunctata unattacked. These individuals dropped from the branch immediately upon encountering an *F. rufa* worker, almost certainly before the ant could respond aggressively. Slightly more *C. septempunctata* were seized by ants, although the proportion of *C. septempunctata* and *C. magnifica* actually seized by *F. rufa* was low and did not differ significantly. All individuals, except one *C. septempunctata* dragged off the colony by *F. rufa* workers, were almost certainly unharmed by *F. rufa*, other seized ladybirds eventually escaping.

Only *C. magnifica* fed upon the tended aphids, although the proportion of *C. magnifica* which were observed feeding was quite low (18%), with the difference significant at only the 5% level (Table III). Under attack, both species defended themselves by pulling their head under their pronotum and drawing their antennae and legs close to their body. They were often observed to roll from side to side. The function of this behavior appeared to be to keep the pronotum and elytra of the ladybird facing the attacking *F. rufa* worker and to expose none of the underside, between the ladybird's elytron and the substrate, on the side being attacked. Such defensive behavior appeared to be of much shorter duration in *C. septempunctata*, which would quickly run or drop from the colony. A feeding *C. magnifica* generally continued to do so while defending itself, although *C. magnifica* adults have also sometimes been observed to carry an aphid away from a tended colony under attack (Sloggett, 1998). Many *C. septempunctata* did not defend themselves at all, but rapidly escaped. The difference in proportions of the two ladybird species using defense is highly significant (Table III).

Differences in the occurrence and duration of defense behavior are manifest in the highly significant difference in time spent on the aphid colony, with *C. magnifica* spending much longer. Some difference in duration on an aphid colony may also accrue from the tendency of *F. rufa* to attack *C. magnifica* somewhat less readily than *C. septempunctata* (see the first section under Results). Reflex bleeding was observed only in *C. septempunctata* individuals which had been seized by ants.

Both species escaped from *F. rufa* attacks or encounters by running from the aphid colony or dropping off the branch. However, *C. magnifica* more often walked or ran away from attack, whereas *C. septempunctata* showed a higher tendency to drop from the branch, a more rapid escape response, this difference in frequencies being significant (Table III). Data based upon the initial frequencies of dropping or running escape behavior certainly underestimate the difference, since four further *C. septempunctata* which initially ran eventually also dropped, whereas only one further *C. magnifica* left the branch, by flying (on the basis of final escape behavior utilized, $X^2 = 12.29$, 1 df, $P < 0.001$).

That *C. magnifica* naturally feeds upon heavily tended aphids, and is attacked by *F. rufa* when doing so, is supported by a number of field observations made during the course of this work. *Coccinella magnifica* was observed at least twice naturally feeding upon heavily tended colonies of aphids on birch, while under attack from *F. rufa*. Similar observations were made several times on pine and once on sporadically tended aphids on rosebay willowherb, *Chamerion augustifolium* (L.). On one occasion a *C. magnifica* adult was observed repeatedly entering a colony of *F. rufa*-tended birch aphids, then retreating, under attack, carrying an aphid in its jaws, which it consumed away from the ants.

DISCUSSION

The results obtained here, with *F. rufa*, almost certainly typify the interactions which occur between *C. magnifica* and all members of the *F. rufa* group. It is probable that *C. magnifica* overrides the degree of *F. rufa* aggression displayed toward *C. septempunctata* through some form of chemical inhibition. This might be some form of chemical mimicry, although extreme chemical repellence seems a more probable explanation (Sloggett, 1998). The nature of *C. mag-*

nifica's chemical inhibition of ant aggression, as well as adult morphological and larval adaptations, will form the basis of later papers in this series.

The degree of *F. rufa* aggression displayed toward *C. magnifica* adults varies with ecological context. On *F. rufa* trails, where *C. magnifica* frequently occurs, the decrease in aggression displayed toward *C. magnifica* compared to that displayed toward *C. septempunctata* is marked. It is less so on colonies of *F. rufa*-tended aphids, where *C. magnifica* feeds. Here *C. magnifica* is more readily attacked by *F. rufa*. Several factors may contribute to this. Ants on foraging trails may only be searching for prey, whereas those on aphid colonies are defending a resource. Although *F. rufa* will prey upon ladybirds (Sloggett, 1998) many species' considerable chemical defenses must make them a poor meal (Happ and Eisner, 1961; Pasteels *et al.*, 1973; Bhatkar, 1982; Marples, 1993; Glisan King and Meinwald, 1996) and defense of aphid prey must surely be the more important to *F. rufa*-group ants, which rely heavily on aphids for honeydew (e.g., Wellenstein, 1952; Jensen, 1978; Skinner, 1980). At a mechanistic level a change in aphid behavior may serve to alert the ants to the presence of a destructive intruder on colonies (Nault *et al.*, 1976). Caste differences between aphid-tending ants, displaying ownership behavior, and other, predatory workers may also be important (Way, 1963).

Given the evidence here of a high level of aggression displayed towards *C. magnifica* by aphid-tending *F. rufa*, it may seem surprising that Majerus (1989) found *C. magnifica* to be unattacked near tended aphids. There are several possible explanations for this difference. All ladybirds used here encountered ants, whereas some of Majerus' ladybirds may have failed to do so. *Formica rufa* aggression can vary with the aphid species being tended. The aphid species, *C. pini*, used here is well defended through effective *F. rufa*-group ant aggression (Völkl and Kroupa, 1997). Majerus used a number of *F. rufa*-tended aphid species on oak, birch, and pine, which may have been less aggressively defended than *C. pini*. The experiments described here were also carried out late in the season, when tended aphids may be scarcer and their importance to *F. rufa* as a resource greater. Earlier in the season there may be an excess of aphids untended by *F. rufa* (Way, 1963; Scheurer, 1971) and *C. magnifica* adults and their soft-bodied larvae may be able to feed without being attacked on untended or sporadically tended colonies. Seasonal differences in *F. rufa* ownership behavior may thus also explain the conflict between these results and those of Majerus (1989). It does, however, seem clear that Majerus was erroneous in his assumption that *C. magnifica* is always immune from *F. rufa* attack.

Away from tended aphids, *C. magnifica*'s putative chemical adaptations appear of more importance than behavior. Differences in the behaviors of *C. magnifica* and *C. septempunctata*, such as reflex bleeding or flying in *C. septempunctata*, may be ascribed to the increased aggression displayed toward this species. *Coccinella magnifica* will readily reflex bleed if alarmed, for example (Donisthorpe, 1903; J. J. Sloggett, personal observation). The characteristic "ducking down" behavior described by Donisthorpe (1900) is also displayed by *C. septempunctata*, even when not attacked by *F. rufa*, and it must be assumed that this was present in *C. magnifica*'s ancestors before their obligate association with the *F. rufa* group evolved.

Behavioral adaptation is of more importance when *C. magnifica* feeds upon colonies of tended aphids. Here chemical adaptation often serves only to delay ant attack, rather than inhibiting it completely, and the ladybird must frequently defend itself. Behavioral modifications of the *C. septempunctata* plan are observed here. *Coccinella magnifica* defends itself more readily than *C. septempunctata*, although in the same manner. It is less prone to drop from the colony, a rapid escape response, and more likely to escape by running away, enabling it to remain in the vicinity of tended aphids when not feeding. In its proper context, it appears that *C. magnifica* has evolved effective defensive behavior to counterattack by aphid-tending *F. rufa*-group ants rather than its having lost any "fleeing response" through lack of usage (Majerus, 1994). *Coccinella magnifica* behavior remains similar, and equally effective, even when *C. magnifica* is exposed to non-*F. rufa*-group ants, which it rarely encounters in the wild (Arnold *et al.*, in Majerus, 1994; Sloggett, 1998).

There appear to be few, if any, behaviors unique to *C. magnifica*. Most *C. magnifica* behavior is modified *C. septempunctata* behavior, with differences occurring in the degree to which behaviors in the repertoire of the two species are expressed. Similar observations have been made by Milbrath *et al.* (1993) in comparing the myrmecophilous chrysopid *Chrysopa slossonae* Banks with its sister species *Chrysopa quadripunctata* Burmeister. Modifications of expression can undoubtedly evolve more easily than totally novel behaviors, through a gradualistic process. Observations that *C. septempunctata* may sometimes interact with *F. rufa*-group ants (Donisthorpe, 1919–1920; Bhatkar, 1982; Sloggett, 1998) provide some evidence of the presence of selective pressures for such evolution to occur. This analysis of behavior, together with Bhatkar's claim that *C. septempunctata* can locate aphids using the pheromone trails of *Formica polyctena* Förster (Bhatkar, 1982), argues that the evolution of *C. magnifica*'s myrmecophily may have been gradualistic. An understanding of the chemical aspect of *C. magnifica*'s aggression avoidance, which is currently being investigated, may help clarify to what extent this was indeed the case.

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