

Ant mutualists alter the composition and attack rate of the parasitoid community for the gall wasp *Disholcaspis eldoradensis* (Cynipidae)

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Abstract. 1. The strength or density dependence of pairwise species interactions can depend on the presence or absence of other species, especially potential mutualists.

2. The gall wasp *Disholcaspis eldoradensis* induces plant galls that secrete a sweet honeydew from their top surfaces while the wasp larvae are active. These galls are actively tended by Argentine ants, which collect the honeydew and drive off parasitoids attempting to attack the gall wasp.

3. When ants were excluded, the total rate of parasitism by seven species of parasitoids increased by 36%, and the rate of gall-wasp emergence decreased by 54%.

4. The total percentage parasitism was affected by gall density when ants were excluded but not when ants were unmanipulated, suggesting a change in parasitoid functional responses due to ant tending.

5. In addition, excluding ants significantly altered the proportions of different parasitoid species that emerged from galls; one parasitoid species increased from 1% to 34%, and another decreased from 46% to 19%.

6. The invasive Argentine ants studied are capable of maintaining the mutualism with the gall wasps that evolved in the presence of different ant species and also act as a selective filter for the local community of generalist parasitoids trying to attack this gall species.

Key words. Ant mutualism, Cynipidae, gall maker, oak, parasitoid community, plant–insect interactions, *Quercus lobata*.

Introduction

Ants participate in a wide variety of mutualisms, including consuming food rewards such as extrafloral nectar on plants, in exchange for providing plants and trophobionts with protection from their natural enemies (see, e.g. Inouye & Taylor, 1979; Fritz, 1983; Huxley & Cutler, 1991; Morales, 2000). The presence of tending ants in a mutualism can strongly affect the nature of pairwise interactions between other species, changing the magnitude or even the sign of an interaction (Messina, 1981; Fritz, 1983; Ito &

Higashi, 1991). An unusual ant–insect mutualism exists between ants and some gall wasps in the family Cynipidae that induce galls and provide a reward for attending ants. Honeydew-secreting galls are found on several species of oaks (*Quercus* spp.) in Europe, Asia, and North America (Bequaert, 1924) and were even described by the ancient Greeks (Theophrastus, translated in Bequaert, 1924). Rather than providing a reward for the ants directly, as do ant-tended homopterans and some lyceanid larvae, these gall-wasp species induce their host oak trees to secrete droplets of a sweet exudate on the surface of the developing gall. This is an impressive feat because oaks are not known to have any floral or extrafloral nectaries.

A few previous studies of ant–cynipid mutualisms have shown that, when ants tending cynipid galls are excluded, the parasitism rate of the gall maker increases (Washburn,

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1984; Abe, 1992; Seibert, 1993; Fernandes *et al.*, 1999). In two of these studies only a single parasitoid species was reported (Abe, 1992; Fernandes *et al.*, 1999), and Washburn (1984) reported the composite parasitism rate. When multiple parasitoids are present, however, all natural enemies are unlikely to be equally deterred by the presence of tending ants (Barzman & Daane, 2001). Seibert (1993) found that ants affected some parasitoid and inquiline species attacking the galls of *Disholcaspis perniciosus* (Cynipidae) more than others. The effects of ant tending on different parasitoid species may also depend on the local density of gall makers, which determines the quantity of honeydew available. The number of tending ants in an area may be limited, and if multiple resources (i.e. various Homoptera or extrafloral-nectar-bearing plants) are available, then gall wasps might compete for ant protection with other insects that secrete honeydew as well as competing intraspecifically (Cushman & Addicott, 1989; Cushman & Whitham, 1991).

At the study site in California, at least seven species of parasitoids attack the galls of *Disholcaspis eldoradensis* Beutenmüller. These cynipid galls are induced in clusters of varying densities (from 1 to >25 galls) along small twigs, are woody, and secrete a sweet honeydew that attracts ants (Weld, 1957; Russo, 1979; Washburn, 1984). In the study reported here, four questions were investigated: (1) Does the presence of tending ants lower parasitoid attack rates? (2) Does the presence of ants alter the parasitoid community, affecting some species of parasitoids more than others? (3) Do the interactions among the gall makers, ants, and parasitoids depend on the number of galls in a cluster or the size of individual galls? (4) Does the effect of tending ants vary spatially (among trees)?

Methods

Eleven trees of *Quercus lobata* Nee (valley oak) growing on the edge of the University of California, Davis, campus (Davis, California) were used. All trees had large numbers of the invasive Argentine ant, *Linepithema humile* Mayr, in active foraging columns on their trunks throughout the experiments. In July 1999 a ring barrier of Tanglefoot (The Tanglefoot Company, Grand Rapids, MI) was applied to each of 41 branches (from two to 10 branches on each tree) and 71 other branches were tagged as controls. Each branch bore a single cluster of 1–24 freshly erupted galls of *D. eldoradensis* along a span of less than 10 cm of the branch. The total number of galls per tree was estimated from a count of all galls that could be found in 3 min of searching. The ants tending the galls during a 1-min interval were counted once per week for 3 weeks as a test of the effectiveness of the Tanglefoot treatment, and any twigs or leaves that acted as bridges were clipped. In October 1999 the galls were collected individually into size '0' gel caps and placed in boxes left under ambient outdoor conditions (Shorthouse, 1972). Emergences were recorded approximately once a week from October 1999 through February 2001, at which time the few remaining galls were cut open; all remaining galls were found to be empty. Vouchers of all

species were placed with the Bohart Museum, University of California, Davis. The lengths of the gall makers and parasitoids were determined with an ocular micrometer to the nearest 0.1 mm, from the front of the head to the end of the abdomen. The abdomen was gently uncurled if necessary. Gall size was measured as the average diameter of the gall along two axes, with a digital calliper. The data were analysed with regression, logistic regression, and analysis of variance (ANOVA) models in S-Plus 6.0 (Insightful Corporation, 2001). Response variables that were percentages were arcsine-square-root transformed so that normality of residuals would be improved.

Results

The Tanglefoot treatment proved effective for keeping ants away from the galls [control 11.3 ± 1.5 (SE) ants per cluster, treatment 0.3 ± 0.1 , $F_{1,78} = 38.71$, $P < 0.0001$]. Most galls on branches with Tanglefoot were not visited by ants, but occasionally a leaf or twig fell so as to make a bridge onto treatment branches and allowed ants temporary access. On the branches from which ants were excluded, flies, wasps, bees, and beetles were observed eating droplets of honeydew, and moulds grew on the top surfaces of a few galls (B. D. Inouye and A. A. Agrawal, unpubl. data). None of the control galls tended by ants became mouldy or were observed being visited by other insects. On control branches the number of ants increased with the number of galls, but the mean number of ants per gall was negatively associated with gall density, decreasing from an average of more than four ants to about one as the number of galls in a cluster increased (Fig. 1). While a linear regression is significant ($F_{1,78} = 5.72$, $P = 0.019$), this relationship is more accurately described as triangular (Fig. 1), due to other factors that also influence ant abundances.

The exclusion of ants from the galls significantly affected the rate of parasitism and the rate of gall-maker emergence (Table 1) ($\chi^2_2 = 18.58$, $P = 0.0001$). Seven parasitoid species were reared from the galls over 11 months: *Ormyrus venustus* Hanson (Ormyridae); *Sycophila* sp. and *Tenuipetiolus* sp. (Eurytomidae); *Torymus californicus*

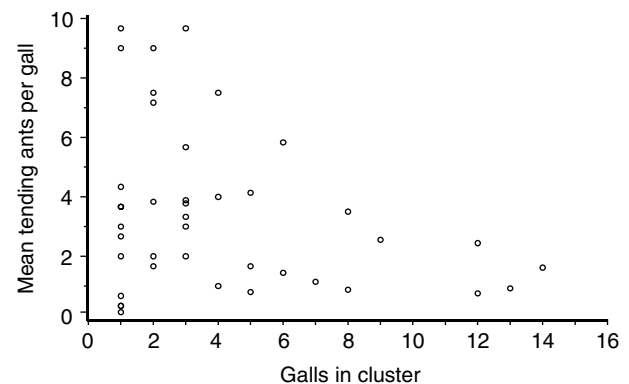


Fig. 1. A general decrease in the mean number of attending ants per gall as the number of galls per cluster increases.

Table 1. Contingency table for the effects of experimental ant exclusion on the fate of *Disholcaspis eldoradensis* galls.

	Successful emergence		Parasitoid emergence		Unknown mortality	
	No.	%	No.	%	No.	%
Ant exclusion	43	23.1	97	52.2	41	22.0
Control	108	43.2	95	38.0	43	17.2

Ashmead, *T. denticulatus* Breland, and *T. warreni* Cockerell (Torymidae); and *Baryscapus* sp. (Eulophidae). In addition to the effect of ant tending on the total rate of parasitism, the composition of the parasitoid community was significantly different on galls where ants were excluded (Table 2) ($\chi^2_4 = 39.12$, $P < 0.0001$). Some species of parasitoids (e.g. *Torymus warreni*) were apparently much more sensitive than others (e.g. *Tenuipetiolus* sp.) to the presence of tending ants, which may be due to some species attacking the gall-makers before the galls are externally visible and thus before ants are recruited. Species of *Torymus* are likely to be idiobionts and facultative hyperparasitoids that attack the galls much later in their development (Hawkins & Goeden, 1984), while ants are more actively tending the galls and after *Sycophila* sp. and *Tenuipetiolus* sp. have attacked galls. Species of *Torymus* may also be facultatively phytophagous (Hawkins & Goeden, 1984), which is consistent with the observation that these species emerged later and at larger adult sizes than all other parasitoids.

With ants present, the number of galls in a cluster affected neither parasitism nor gall-wasp emergence rates, but when ants were absent, both were significantly affected; more parasitoids emerged per gall in larger clusters (Table 3). A quadratic term for the number of galls was included as a test for a possible effect of the treatment on the curvature of the summed parasitoid functional response, as opposed to a change in the slope of a linear relationship. The significant interaction between the treatment and the squared number of galls in a cluster indicates that the summed parasitoid functional response to local host density was significantly altered by the presence of tending ants. The parasitoid attack rate summed over all parasitoid species was similar in shape to a Holling type II (saturating) functional response when ants were present and

was nearly linear when ants were absent (Fig. 2). Because only one cluster of more than 14 galls was found, the results were also analysed without that cluster. When that data point was excluded the quadratic term was no longer significant, but a significant linear interaction was apparent between the number of galls in a cluster and the treatment (galls \times treatment, $F_{1,106} = 7.80$, $P = 0.006$). Although total parasitism was related to the local density of hosts, measured as the number of galls in a cluster, it was not significantly related to the total number of galls counted on a tree in any analysis (all $P > 0.23$).

The average gall diameter was 6.05 mm (SD = 1.2 mm), and average gall size decreased slightly as the number of galls in a cluster increased ($F_{1,96} = 4.28$, $P = 0.041$). Individual gall size had no effect on total parasitism rate (logistic regression, $t = 1.27$, $P = 0.214$), but on average, different species of parasitoids came from different sizes of galls ($F_{5,164} = 14.73$, $P < 0.0001$); larger species (e.g. *Torymus* spp.) tended to emerge from larger galls. Trees differed marginally significantly in the effect of the ant-exclusion treatment on the parasitoid attack rate (tree-by-treatment interaction: $F_{10,90} = 1.90$, $P = 0.055$). Galls from different trees also produced different proportions of parasitoid species, but the sample sizes were too low for a rigorous statistical test.

Discussion

Where ants were excluded, the total parasitism rate of *D. eldoradensis* galls increased and gall-maker emergence declined. This pattern is consistent with a mutualism between ants and *D. eldoradensis* galls. This kind of mutualism has been suggested by previous studies on ant-tended cynipid galls (Washburn, 1984; Abe, 1992; Seibert, 1993; Fernandes *et al.*, 1999), but in the study reported here, both the composition of the parasitoid community emerging from galls and the pattern of attack rates of the total parasitoid community depended strongly on ant tending. These patterns have not been reported from earlier studies, although in all but one case (Fernandes *et al.*, 1999), multiple parasitoid species were reared from the galls.

It is interesting to find effects on the parasitoid community of tending by Argentine ants, which are not native to California but have largely displaced native ants from much

Table 2. Differential responses of parasitoids to ant exclusion from *Disholcaspis eldoradensis* on valley oaks (number of individuals followed by percentage within each treatment). For *Baryscapus* sp. the data are the number of galls from which these parasitoids emerged rather than the number of individuals, because as many as 21 wasps emerged from a single gall.

	Parasitoid species									
	<i>Sycophila</i> sp.		<i>Tenuipetiolus</i> sp.		<i>Baryscapus</i> sp.		<i>Torymus warreni</i>		All others	
	No.	%	No.	%	No.	%	No.	%	No.	%
Ant exclusion	17	18.9	13	14.4	19	21.1	31	34.4	10	11.1
Control	40	46.0	13	14.9	28	32.2	1	1.1	5	5.7

Table 3. ANOVA table for the effects of the ant-exclusion treatment, number of galls in a cluster, and the number of galls² on number of emerging parasitoids.

Factor	d.f.	Sum of squares	Mean square	F	P
Number of galls	1	41.46	41.46	26.43	<0.0001
Treatment	1	0.04	0.044	0.03	0.867
Galls ²	1	0.02	0.020	0.01	0.909
Galls by treatment	1	0.48	0.484	0.31	0.579
Treatment by galls ²	1	6.22	6.222	3.96	0.049
Residuals	106	166.27	1.568		

of the state. The mutualism between native ants and *D. eldoradensis* has apparently been usurped by Argentine ants, but in other interactions on trees with native ants, mutualisms with scale insects and a commensalism with another cynipid gall wasp, Argentine ants do not fill the same role as native ants (B.D. Inouye, unpublished manuscript).

Density dependence in parasitism and gall-maker emergence was contingent on the lack of tending ants; the presence of ants eliminated this effect. In this system ant tending seems generally effective at reducing parasitism, even though few ants tend each gall in dense clusters. The amount of available sugar per twig may therefore be a limiting resource, and the average quality of the exudate reward for the ants may therefore decline as the number of galls in a cluster increases. A decrease in the number of ants per honeydew producer with an increase in the number of honeydew producers has also been observed by Breton and Addicott (1992) and Morales (2000).

When ants were excluded, parasitoid foraging was affected by gall density. Abe (1992) found the opposite result in experiments with *Andricus symbioticus* (Cynipidae); gall cluster density did not affect parasitism when ants were absent, but in the presence of ants gall

density and parasitism were negatively associated. The difference may be due to different behaviours of the tending ant species, because, in contrast to the results of the work reported here, ants abandoned some small clusters of *A. symbioticus* galls. No effect was found of gall diameter on total per cent parasitism or per cent gall-maker emergence, but on average different parasitoid species emerged from different sizes of galls, with the largest species (*Torymus* spp.) emerging from the largest galls.

Although parasitism was affected by the number of galls in a cluster, no evidence of density-dependent parasitism at the scale of entire trees was found, despite the observation that individual trees tend to have similar densities of galls in different years (B.D. Inouye and A.A. Agrawal, unpublished data). The marginally significant interaction between the ant-exclusion treatment and tree identity may be due to spatial variation in the number of ants or variation among trees in the average size of gall clusters on each twig rather than to the total number of galls on a tree.

The patterns of parasitoid attack rate (summed over the seven-species parasitoid assemblage) with and without ants are different (on the basis of the significant galls² × treatment interaction in Table 3). The significance of this interaction is strongly affected by a single large cluster of galls from which ants were excluded. If this point is excluded the quadratic term is no longer significant, but the linear interaction (galls × treatment; Table 3) then becomes significant instead. This result still indicates a difference between treatments in the pattern of density-dependent parasitoid attacks. This finding supports the idea that host–parasitoid interactions depend on the community context (Hawkins, 1994). Thus, variation in local abundance or recruitment of ants could lead to spatial variation in parasitoid functional responses. Unfortunately, the data set is not large enough to test the functional responses of individual parasitoid species, but in the absence of any systematic relationship between parasitoid densities and treatments, which were interspersed within each tree, the change in total parasitism rates logically indicates that at least some of the individual species' functional responses responded to ant tending. This kind of spatial variation in parasitoid functional response may be stabilising for host–parasitoid population dynamics when a single parasitoid species attacks a host (Holt & Hassell, 1993), but the consequences for a multispecies parasitoid community are unexplored.

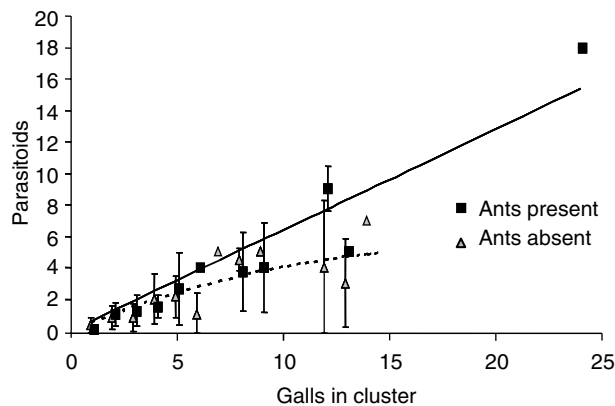


Fig. 2. The mean number of parasitoids emerging from gall clusters of differing size and fitted lines from ANOVA. Triangles represent the ant-exclusion galls, and squares represent control galls with attending ants. The data points have been shifted slightly to reduce overlap, and the error bars show one standard error for each point.

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References

- Abe, Y. (1992) The advantage of attending ants and gall aggregation for the gall wasp *Andricus symbioticus* (Hymenoptera: Cynipidae). *Oecologia*, **89**, 166–167.
- Barzman, M.S. & Daane, K.M. (2001) Host-handling behaviours in parasitoids of the black scale: a case for ant-mediated evolution. *Journal of Animal Ecology*, **70**, 237–247.
- Bequaert, J. (1924) Galls that secrete honeydew. *Bulletin of the Brooklyn Entomological Society*, **19**, 101–124.
- Breton, L.M. & Addicott, J.F. (1992) Density-dependent mutualism in an aphid–ant interaction. *Ecology*, **73**, 2175–2180.
- Cushman, J.H. & Addicott, J.F. (1989) Intra- and interspecific competition for mutualists: ants as a limited and limiting resource for aphids. *Oecologia*, **79**, 315–321.
- Cushman, J.H. & Whitham, T.G. (1991) Competition mediating the outcome of a mutualism – protective services of ants as a limiting resource for membracids. *American Naturalist*, **138**, 851–865.
- Fernandes, G.W., Fagundes, M., Woodman, R.L., Price, P.W. (1999) Ant effects on three-trophic level interactions: plant, galls, and parasitoids. *Ecological Entomology*, **24**, 411–415.
- Fritz, R.S. (1983) Ant protection of a host plant's defoliator: consequence of an ant–membracid mutualism. *Ecology*, **64**, 789–797.
- Hawkins, B.A. (1994) *Pattern and Process in Host–Parasitoid Interactions*. Cambridge University Press, Cambridge.
- Hawkins, B.A. & Goeden, R.D. (1984) Organization of a parasitoid community associated with a complex of galls on *Atriplex* spp. in southern California. *Ecological Entomology*, **9**, 271–292.
- Holt, R.D. & Hassell, M.P. (1993) Environmental heterogeneity and the stability of host–parasitoid interactions. *Journal of Animal Ecology*, **62**, 89–100.
- Huxley, C.R. & Cutler, D.E. (1991) *Ant–Plant Interactions*. Oxford University Press, New York.
- Inouye, D.W. & Taylor, O.R.J. (1979) A temperate region plant–ant–seed predator system: consequences of extra floral nectar secretion by *Helianthella quinquinervis*. *Ecology*, **60**, 1–7.
- Insightful Corporation (2001) *S-Plus 6.0*. Insightful Corporation, Seattle, Washington.
- Ito, F. & Higashi, S. (1991) An indirect mutualism between oaks and wood ants via aphids. *Journal of Animal Ecology*, **60**, 463–470.
- Messina, F.J. (1981) Plant protection as a consequence of an ant–membracid mutualism: interactions on goldenrod (*Solidago* sp.). *Ecology*, **62**, 1433–1444.
- Morales, M.A. (2000) Mechanisms and density dependence of benefit in an ant–membracid mutualism. *Ecology*, **81**, 482–489.
- Russo, R.A. (1979) *Plant Galls of the California Region*. Boxwood Press, Pacific Grove, California.
- Seibert, T.F. (1993) A nectar-secreting gall wasp and ant mutualism: selection and counter-selection shaping gall wasp phenology, fecundity, and persistence. *Ecological Entomology*, **18**, 247–253.
- Shorthouse, J.D. (1972) An emergence technique for small insects. *Canadian Entomologist*, **104**, 1131–1132.
- Washburn, J.O. (1984) Mutualism between a cynipid gall wasp and ants. *Ecology*, **65**, 654–666.
- Weld, L.H. (1957) *Cynipid Galls of the Pacific Slope*. Privately printed, Ann Arbor, Michigan.

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