Ant tending influences soldier production in a social aphid

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The aphid *Pseudoregma sundanica* (Van der Goot) (Homoptera: Aphididae) has two defence strategies. It is obligatorily tended by various species of ant and also produces sterile soldiers. We investigated how they allocate their investment in these two strategies. We measured the size, number of soldiers, number and species of tending ant, and number and species of predators in *P. sundanica* populations. We found that the level of ant tending correlated negatively with soldier investment in *P. sundanica*. The species of tending ant also influenced soldier investment. We excluded ants from aphid populations and recorded changes in population size and structure over four weeks. Ant exclusion led to population decline and extinction. At the same time, surviving populations showed a significant increase in soldier investment. The data demonstrate that social aphids can adjust their investment in soldiers in direct response to environmental change.

Keywords: aphid; ant; soldiers; polymorphism; defence investment; *Pseudoregma sundanica*

1. INTRODUCTION

Defence against predation is generally assumed to impose costs (Maynard Smith 1972). Evolution should act to optimize investment in defence by balancing these costs with the benefits (Rhoades 1979). Theoretical studies have shown that defence investment will be influenced by environmental factors such as predation levels, innate factors such as defence efficacy and the availability of alternative forms of defence (Oster & Wilson 1978; Harvell 1990; Lima & Dill 1990; Endler 1991; Stern & Foster 1996). Theories of defence optimization are most easily tested in systems where organisms potentially show a graded investment in response to a spatially or temporally variable environment. Social insects, where there are defensive and non-defensive individuals, are such a system. The defence investment of a colony will be related to the proportion of defensive individuals in that colony (Oster & Wilson 1978; Wilson 1985). Of particular interest are the parthenogenetic soldier-producing aphids where there is no genetic conflict over caste ratios within a clone.

Soldier production occurs in several aphid species of the families Pemphigidae and Hormaphididae (Stern 1994). These soldiers are typically modified early instars, which are specialized for defence and which engage in confrontation with potential predators. The modification is always ethological and is often morphological. Because they have an increased risk of mortality and development to the next instar can be delayed or not occur at all (in the case of sterile soldiers), it is reproductively costly for an aphid clone to produce soldiers (Stern & Foster 1996). Soldier production should therefore reflect environmental conditions as predicted by optimization theory.

There have been few empirical studies on the forces that influence soldier investment in aphids. A decrease in the proportion of soldiers in a population has been correlated with a decrease in population size (Sakata *et al.* 1991; Schütze & Maschwitz 1991; Itô *et al.* 1995), predator

abundance (Shibao 1998), gall size (Stern *et al.* 1994) and colony age (Akimoto 1992) and an increase in the age of host plants (Sakata *et al.* 1991). Seasonal variations have also been recorded (Sunose *et al.* 1991). However, experimental manipulation of these factors has not yet been shown to influence soldier production directly. Cues that influence allocation in morphs other than soldiers have been better studied. For example, winged-aphid production can be suppressed by ant tending (Banks 1958) and triggered by increased crowding (Dixon 1998), predation (Weisser *et al.* 1999) and changes in day length (Matsuka & Mittler 1978). Finally, intraspecific competition has been shown to alter defensive caste allocation in ants (Passera *et al.* 1996).

One potential environmental influence of aphid soldier investment that has yet to be fully investigated is ant tending. Many aphids are involved in a symbiotic interaction with ants (myrmecophily). Aphids defecate honeydew, the sweet waste product of their sugar-rich but amino-acid-poor diet of plant sap. Honeydew can be an important source of sugar for the ants and so they may defend the aphids from natural enemies (Banks 1962). Whilst there is a paucity of evidence for clearly demonstrating the role ants play in aphid defence, ant tending is generally considered to confer gross benefits on aphids (Way 1963). It may also confer costs. Ants may feed on the aphids they tend in excess of those needed to satisfy honeydew requirements (Rösengren & Sundstrom 1991; Sakata 1994). Aphids may also adjust their honeydew quantity or composition in order to encourage ant tending, at a cost to themselves (Del-Claro & Oliveira 1993; Stadler & Dixon 1998).

Assuming aphids optimize investment in defence, ant tending should influence or be influenced by soldier production within an aphid population. The precise nature of the interaction will not only be determined by the relative costs and benefits of each strategy, but whether the aphids can control their investment in each. The interaction between myrmecophily and soldier production has not been well studied. Schütze & Maschwitz (1991) looked at the behaviour of ant-tended *Pseudoregma*

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sundanica soldiers. They found that the soldiers attacked predators not attacked by ants. Sakata *et al.* (1991) observed that ants visited small, soldierless colonies of the related South-East Asian *Pseudoregma bambucicola* (Takahashi), whilst larger colonies with numerous soldiers were rarely visited by ants.

Our aim was to investigate defence allocation in aphids by elucidating the relationship between myrmecophily and soldier production. In particular, we wished to determine whether soldier production can be manipulated through changes in the level of ant tending or vice versa by collecting observational and experimental data.

2. METHODS

(a) *Study organism*

The aphid *P. sundanica* feeds on the stem, bases of the leaves and, occasionally, undersides of the leaves of various species of ginger (Zingiberaceae). It is an obligate myrmecophile. *P. sundanica* also produces soldiers. These are sterile first instars that differ from their 'normal' siblings in their defensive behaviour, which involves hind leg waving, possibly to ward off flying predators and grasping with the forelegs when attacked (Schütze & Maschwitz 1991). Their abdomens are more elongated than in normal first instars and they develop heavily sclerotized forelimbs and extended frontal horns. However, newborn, unsclerotized soldiers are morphologically very similar to newborn, normal first instars under a hand lens (A. W. Shingleton, personal observation).

(b) *Field sites*

The study was conducted in peninsular Malaysia. There were two sample sites: (i) the Ulu Gombak Valley, 30 km north-east of Kuala Lumpur, and (ii) the Cameron Highlands, 200 km north of Kuala Lumpur and 50 km east of the Straits of Malacca. All the data were collected between January and March 1999.

(c) *Census*

We conducted a census of 150 *P. sundanica* populations from both sites (a population includes all aphids of the same species living on a single host plant). Each population was censused once only. For each population we recorded the following information.

- (i) The number of normal aphids on the host plant (adults and juveniles).
- (ii) The number of soldier aphids.
- (iii) The number of any tending ants.
- (iv) The number and species of any predators.

We inspected populations with a hand lens and used morphology for distinguishing between soldiers and non-soldiers. This may have underestimated the actual number of soldiers as it did not include those soldiers too young to have fully developed soldier morphology. We took samples of ants from 140 of the 150 populations for later identification under a microscope. We identified these ants to genus using a key (Bolton in Hölldobler & Wilson 1990) and then morphotyped species within each genus.

(d) *Experiment 1: ant exclusion*

In order to establish how myrmecophily affects soldier production, ants were excluded from 31 populations of *P. sundanica* in the

Ulu Gombak Valley. The populations ranged in size from six to 2543 individuals and were tended by a variety of ant species. Ants were excluded using Tanglefoot™ applied to waterproof sticky tape wrapped around the base of the host plant. The populations were inspected twice a week in order to ensure no ants breached the defences. We counted the number of normal and soldier aphids on each host plant once a week. We also counted the number of first-instar 'leg wavers' in each population. This count included all distinguishable soldiers as well as those defensive juveniles too small to be classified as soldiers or non-soldiers using a hand lens. We induced leg waving by blowing lightly on the aphids. The experiment lasted four weeks. Ants were reintroduced to two populations after the end of the experiment and the numbers of normal and soldier aphids were counted after 24–30 h. As a control, 11 populations were treated identically, except that Tanglefoot™ was not applied to the sticky-tape band.

(e) *Experiment 2: soldier behaviour*

In order to determine whether only soldiers were involved in defensive behaviour, we induced leg waving in 17 populations by blowing gently on the aphids. Up to 20 leg wavers were collected from each population and inspected under a dissecting microscope.

(f) *Statistical analysis*

Statistical analyses were performed using Minitab® 12.1. We tested most of the data using the general linear model (GLM). This allowed us to assess the relationship between a single variable and multiple factors and test the influence of each factor independently whilst controlling for the effects of the others. The model was specified for each test. The data were Box–Cox transformed in all parametric statistical tests, unless stated otherwise, so that they did not deviate significantly from normality, linearity or constant variance (Sokal & Rohlf 1995). The data for figures were log or arcsine-square-root transformed for clarity but statistically unadjusted for other variables. Significance was taken at $p < 0.05$.

3. RESULTS

(a) *Census*

With a single exception, all 150 *P. sundanica* populations were found with tending ants. We identified the ants tending 140 of these populations, which were of 21 different species in nine genera (table 1). Only two of these species were found tending at both sample sites: one of these was found tending a single population with soldiers. Only 30 of the censused populations had soldiers. Table 2 shows the results of the GLM analyses of these populations.

The data indicate that soldier investment decreased with an increase in the level of tending. The proportion of soldiers in a population was negatively correlated with the number of ants per aphid (figure 1 and table 2). The level of tending was itself negatively correlated with population size (table 2). Consequently, larger populations, which received lower levels of tending, produced higher proportions of soldiers (GLM, proportion of soldiers = population size + species of ant + sample site: $F_{\text{population size } 1,17} = 6.74$ and $p < 0.05$). This relationship was only a corollary of the changes in the level of ant tending. Population

Table 1. *Species list of ants found tending P. sundanica with census results*

(Absolute values are given for the mean ants per aphid and mean log population size for species tending only a single population. UGV, Ulu Gombak Valley; CH, Cameron Highlands.)

species size	site: UGV or CH	number of populations	number of populations with soldiers	mean ants per aphid ($\pm$ s.e.)	mean log population size ($\pm$ s.e.)
<i>Iridomyrmex</i> sp. i	UGV	17	5	1.077 $\pm$ 0.482	3.858 $\pm$ 0.410
<i>Iridomyrmex</i> sp. ii	CH	2	1	1.100 $\pm$ 0.177	4.991 $\pm$ 0.584
<i>Iridomyrmex</i> sp. iii	UGV	2	0	0.477 $\pm$ 0.315	3.394 $\pm$ 0.216
<i>Iridomyrmex</i> sp. iv	CH	7	0	1.689 $\pm$ 0.200	4.063 $\pm$ 0.262
<i>Anopleopsis</i> sp.	UGV	7	2	0.292 $\pm$ 0.092	4.331 $\pm$ 0.857
<i>Camponotus</i> sp.	UGV	10	0	0.450 $\pm$ 0.101	2.895 $\pm$ 0.395
<i>Technomyrmex</i> sp. i	both	4	1	1.409 $\pm$ 0.663	3.578 $\pm$ 0.440
<i>Technomyrmex</i> sp. ii	both	6	0	0.592 $\pm$ 0.188	3.202 $\pm$ 0.622
<i>Technomyrmex</i> sp. iii	UGV	1	0	0.600	3.689
<i>Crematogaster</i> sp. i	UGV	17	2	0.567 $\pm$ 0.120	2.600 $\pm$ 0.484
<i>Crematogaster</i> sp. ii	UGV	1	0	0.500	2.079
<i>Crematogaster</i> sp. iii	UGV	1	0	0.500	1.792
<i>Crematogaster</i> sp. iv	UGV	2	1	0.753 $\pm$ 0.347	4.235 $\pm$ 1.932
<i>Crematogaster</i> sp. v	UGV	1	0	0.417	2.485
<i>Crematogaster</i> sp. vi	CH	12	5	0.403 $\pm$ 0.115	4.647 $\pm$ 0.492
<i>Meranoplus mucronatus</i>	UGV	9	2	0.826 $\pm$ 0.190	3.313 $\pm$ 0.699
<i>Oecophylla smaragdina</i>	UGV	9	3	0.180 $\pm$ 0.106	4.810 $\pm$ 0.756
<i>Pheidole</i> sp. i	UGV	4	3	0.112 $\pm$ 0.080	5.262 $\pm$ 0.395
<i>Pheidole</i> sp. ii	UGV	26	4	1.057 $\pm$ 0.124	3.497 $\pm$ 0.331
<i>Pheidole</i> sp. iii	UGV	1	0	0.714	2.639
<i>Myrmecaria</i> sp.	UGV	1	1	0.048	5.517

Figure 1. The relationship between the level of tending (ants per aphid) and soldier investment (proportion of soldiers) in soldier-producing *P. sundanica* populations unadjusted for population size or species of tending ant.

size had no influence on soldier proportion when controlling statistically for the affects of ant per aphid (table 2).

The species of tending ant was also important. Different ant species tended at different levels within each sample site (table 2). Consequently, ant species that showed a high mean level of tending were associated with populations with a low mean proportion of soldiers and vice versa (correlation, $r_{\text{mean ants per aphid for a species, mean proportion of soldiers}} = -0.704$, d.f. = 10 and $p < 0.05$) (absolute values were used for species tending a single population of aphids). Different ant species did not tend populations of different sizes (table 2). However, ants had an additional effect that was independent of their level of

tending. Different species of ant were associated with different proportions of aphid soldiers even when controlling statistically for differences in their level of tending (table 2).

The proportion of soldiers and the level of tending differed between sample sites, but population size did not (table 2). It is not possible to say whether these differences were the result of different ant species tending at each sample site or some other factor.

Predation was extremely low in all of the populations. Only six out of the 150 populations inspected had predators. These were either the larvae of miletine lycaenids (Lepidoptera) or the larvae of a coccinellid (Coleoptera). They were not attacked by the tending ants. All produced a droplet of liquid when palpated by the ants. The ants collected this droplet and this action appeared to prevent further, more aggressive attention. Such a small sample size precludes statistical analysis of any relationship involving predation levels.

(b) *Experiment 1: ant exclusion*

Ant exclusion stimulated an increase in defensive behaviour and soldier production in *P. sundanica* populations (figure 2). The numbers and proportion of leg-waving aphids in the ant-excluded populations increased significantly within one week, despite a decrease in population size. By week 2, the numbers and proportion of distinguishable soldiers were also significantly higher than in week 0. In contrast, there were no changes in the numbers or proportion of soldiers in the ant-tended control populations.

Significantly more of the ant-excluded populations became extinct over the four-week experiment. Only ten

Table 2. *Outputs of GLM analyses of the census data from soldier-producing populations*

(Ant species was nested within sample site because different species of ant tended at each site. Signs in parentheses preceding covariant factors indicate the direction of correlation. ns, not significant. * $p < 0.05$, ** $p < 0.01$.)

source of variance	d.f.	<i>F</i>	<i>p</i>
soldier proportion			
(-) ants per aphid	1	9.58	0.007**
(-) population size	1	0.04	0.842 ^{ns}
ant species	10	2.92	0.027*
sample site	1	18.84	0.001**
error	16	—	—
ants per aphid			
(-) population size	1	5.62	0.031*
(-) soldier proportion	1	9.58	0.007**
ant species	10	4.14	0.006**
sample site	1	9.60	0.007**
error	16	—	—
population size			
(-) ants per aphid	1	5.62	0.031**
(-) soldier proportion	1	0.04	0.842 ^{ns}
ant species	10	1.19	0.364 ^{ns}
sample site	1	0.15	0.701 ^{ns}
error	16	—	—

out of 31 ant-excluded populations survived four weeks compared to eight out of the 11 control populations (Fisher's exact test $p < 0.05$). There was a significant decrease in population size in the ten ant-excluded populations that survived four weeks (figure 3). There was a significant increase in the size of the surviving control populations over the same period.

There appeared to be two causes of population decline and extinction.

- Predation. Nine out of the 31 ant-excluded populations had predators at some stage during the experiment compared to only one out of the 11 control populations, although this was not a significant difference (Fisher's exact test $p = 0.25$). Syrphid (Diptera) and chrysopid (Neuroptera) larvae as well as coccinellid and lycaenid larvae attacked the untended populations. The tended populations (in both the control and the census) were only attacked by coccinellid and lycaenid larvae.
- Migration. Some aphids were found trapped in the Tanglefoot in all of the ant-excluded populations. In two cases these aphids appeared to constitute the entire population.

The reintroduction of ants did not influence the number of soldiers or non-soldiers after 24–30 h in either of the populations where the Tanglefoot™ was removed ($\chi^2_1 = 0.563$ and $p = 0.44$ and $\chi^2_1 = 0.091$ and $p = 0.76$, respectively).

(c) Experiment 2: soldier behaviour

Out of the 232 leg wavers collected, eight were non-soldiers (including two first instars), 163 were soldiers and

Figure 2. Changes in the mean (± s.e.) proportion and number of soldiers in *P. sundanica* populations surviving four weeks after ant exclusion and control populations. There was a significant change in both the number (a) ($\log(T+1)$ transformed) and proportion (b) (arcsine-square-root transformed) of leg wavers and soldiers in ant-excluded populations (GLM, number or proportion of leg wavers or soldiers = week of experiment + population size + population identity: $F_{\text{week } 4,35} > 3.28$ and $p < 0.022$ for all). b, d, f and h are significantly larger than a, c, e and g, respectively (Tukey's simultaneous test, $t_{9,9} > 3.16$ and $p < 0.05$ for all). There was no change in either the number (a) or proportion (b) of soldiers in the control populations (GLM, number or proportion of leg wavers or soldiers = week of experiment + population size + population identity: $F_{\text{week } 4,30} < 1.99$ and $p > 0.12$ for both). Week of experiment was treated as a discrete variable.

61 were first instars that were too juvenile to be classified based on morphology.

4. DISCUSSION

The results show that ant tending influences soldier production in *P. sundanica*. The negative correlation between the number of ants per aphid and soldier proportion suggests that populations invest more in soldiers when ant tending and, presumably, ant defence is low. Ant exclusion led to an increase in the production of soldiers in a population. Populations tended by different species of ant produced different proportions of soldiers. This is the first time, to our knowledge, any factor has been shown to influence soldier production in a social aphid directly.

Ants defend the aphids they tend. *P. sundanica* was almost never found without tending ants and ant exclusion led to population decline and extinction. The ants

Figure 3. Changes in the mean ($\pm$ s.e.) size of *P. sundanica* populations surviving four weeks after ant exclusion and control populations. There was a significant decrease in the size of ant-excluded populations (GLM, population size = week of experiment + population identity: $F_{\text{week } 1,35} = 28.54$ and $p < 0.001$). There was a significant increase in the size of the control populations (GLM, population size = week of experiment + population identity: $F_{\text{week } 1,30} = 6.76$ and $p < 0.05$). Week of experiment was treated as a continuous variable.

actively remove certain predators, such as syrphid and chrysopid larvae (Schütze & Maschwitz 1991). These predators only attacked the aphids once the ants had been removed.

The correlation between the level of ant tending and the proportion of soldiers could result from ants removing soldiers. The total sugar and sugar per honeydew droplet excreted by soldiers of *Pseudoregma koshunensis* is less than that excreted by normal first-instar nymphs (Arakaki & Hattori 1998). Ants may therefore be removing less productive individuals (Sakata 1994). However, ants did not remove soldiers when reintroduced to populations from which they had been excluded. This suggests that soldier proportion is determined by maternal manipulation. In addition, ants should only prey on aphids when they are providing honeydew in excess of the requirements of the ant colony (Rösgren & Sundstrom 1991). This will be associated with a high number of aphids per ant (or a low number of ants per aphid) (Sakata 1994), yet this is when soldier proportions are at their highest. Nevertheless, it is possible that differential predation on *P. sundanica* by ants may in part influence the relationship between the level of ant tending and soldier proportion. Alternatively, ants may avoid populations with a high proportion of soldiers. Large soldier-producing populations of *P. bambucicola* are avoided by ants, despite producing a large amount of honeydew (Sakata *et al.* 1991). However, in *P. sundanica* the absolute number of ants per population increased with population size, suggesting it is the lower level of tending that stimulates soldier production and not vice versa.

The argument that ant tending affects soldier production directly was strengthened by the ant-exclusion experiment. Removing tending ants had a clear and rapid effect on caste structure. The number and proportion of defensive leg-waving aphids increased significantly

within one week. Within two weeks, the number and proportion of identifiable soldiers also increased significantly. The changes in caste proportion may have been partially due to the more rapid death of non-defensive over defensive individuals. However, the absolute increases in the numbers of both leg wavers and soldiers demonstrated that ant exclusion influenced production and not just proportion. We cannot exclude the possibility that the initial leg wavers were not all soldiers, although soldier numbers subsequently increased. Leg-waving behaviour has been recorded in non-soldier *Pseudoregma alexanderi* and *P. bambucicola* (Aoki *et al.* 1981; Sakata *et al.* 1991). However, out of 171 identifiable leg wavers collected from the *P. sundanica* populations, only eight were non-soldiers and only two were normal first instars. The nature of the juveniles, which were too small to be classified with a dissecting microscope, requires further investigation.

The species of tending ant also influenced soldier proportion. Different ant species tended at different levels and the aphids appeared to respond to this by altering their caste allocation. Ant species also affected caste ratios independent of their level of tending. This may result from different species showing different efficiencies at removing predators or at tending aphids, which may in turn be reflected in the aphids' soldier production.

The explanation relating tending levels to soldier production requires that we see more predation when ant tending is low. The census data did not demonstrate this. Predation even by specialized predators was low suggesting that predation acts on a wider spatial or temporal scale than we measured. Further study of the relationship between predator levels and ant tending is clearly needed.

It is not possible to draw any conclusions about the relative costs of soldier versus ant defence. The data are consistent with the hypothesis that ant tending is less costly than soldier production, with aphids investing in soldiers only when ant tending is low. However, if aphids are unable to avoid or control the attention of ants then we would see the same relationship even if ant tending were more costly. Ant tending appears to be the principal form of defence in *P. sundanica*. Small populations can survive without soldiers but they cannot survive without ants and larger populations showed, at the least, a decline when ants were removed, despite soldier defence. Investment in ant defence could therefore be immutable. The aphids may not be balancing investment in myrmecophily against investment in soldiers. Changes in soldier proportion may occur solely because of ant-driven changes in the level of defence the ants provide. If this were the case then the relationship between the level of ant tending and soldier proportion should be independent of the cost of ant tending. Further investigations into the costs and benefits of both forms of defence are required, at both the individual and population levels. Comparisons with a closely related but untended species would be useful.

Ant exclusion leads to a rapid change in caste structure in *P. sundanica*. Given that the age of first reproduction is *ca.* 12 days (A. W. Shingleton and W. A. Foster, unpublished data), this shift was within one generation, suggesting that ant tending acts directly on the reproductive schedule of a mother. In the pea aphid (*Acyrthosiphon pisum*) the introduction of predators initiated the

production of winged morphs within three days (Weisser *et al.* 1999). In general, a rapid direct response in defence investment to changes in an environmental factor will be advantageous if that environmental factor fluctuates rapidly and unpredictably (Harvell 1990). The level of ant tending potentially fluctuates rapidly. One ant species can often usurp another from tending a population of *P. sundanica* (A. W. Shingleton, personal observation), resulting in swift changes in the level of tending that the population receives. It would therefore be advantageous for *P. sundanica* to measure ant tending directly and show a short response time in its resulting caste allocation. The level of ant tending will also be unpredictable. A newly established *P. sundanica* population will probably have no control over the species of ant that tends it and, thus, cannot anticipate the level of tending it receives. Adjustments in defence investment should therefore be made flexibly over ecological time.

Considerably more work needs to be done on *P. sundanica* and other aphids in order to elucidate fully the proximate mechanisms that are responsible for the production of soldiers. In general, an understanding of the way influencing factors vary in space and time will be fundamental to understanding how organisms optimize investment in defence. The importance of environmental factors during evolutionary time is clear. Their influence on defence investment in ecological time is only now becoming apparent.

We thank C. Braendle, N. Pike, N. Rynearson and D. Stern for their discussions and for critical comments on early drafts of the manuscript. We thank H. Disney for his assistance with ant identification. We thank L. Kirton and U. Maschwitz for their assistance during our stay in Malaysia. We thank the Government of Malaysia for permission to conduct research in Malaysia (UPE reference 40/200/19 SJ.627) and the University of Malaysia, Kuala Lumpur, for the use of their field station. Helpful comments came from two anonymous reviewers. A. W. Shingleton is supported by the Biotechnology and Biological Sciences Research Council.

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