Macroevolutionary patterns in the origin of mutualisms involving ants

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Abstract

Ants are a diverse and abundant insect group that form mutualistic associations with a number of different organisms from fungi to insects and plants. Here we use a phylogenetic approach to identify ecological factors that explain macroevolutionary trends in the mutualism between ants and honeydew-producing Homoptera. We also consider association between ant-Homoptera, ant-fungi and ant-plant mutualisms. Homoptera-tending ants are more likely to be forest dwelling, polygynous, ecologically dominant and arboreal nesting with large colonies of $10^4 – 10^5$ individuals. Mutualistic ants (including those that garden fungi and inhabit ant-plants) are found in under half of the formicid subfamilies. At the genus level, however, we find a negative association between ant-Homoptera and ant-fungi mutualisms, while there is a positive association between ant-Homoptera and ant-plant mutualisms. We suggest that species can only specialise in multiple mutualisms simultaneously when there is no trade-off in requirements from the different partners and no redundancy of rewards.

Introduction

Mutualisms are common, fundamental interactions shaping species' communities and, through coevolutionary adaptation, even changing species themselves (Herre et al., 1999). Within a family that contains mixtures of mutualistic and non-mutualistic species we can identify ecological factors that are associated with mutualism. These factors may be preadaptations that predispose a lineage to mutualism or they may be the selective consequences of mutualistic coevolution. Mutualisms are not always stable over evolutionary time and may be lost and gained repeatedly (Sachs & Simms, 2006). If a clade is involved in several different types of mutualism, these mutualisms may be positively or negatively associated or show no association at all.

The ants (Family: Formicidae) engage in a broad range of mutualistic interactions with many different organisms including plants, insects and fungi (Hölldobler & Wilson, 1990). This diversity of mutualistic interaction may reflect the widespread abundance and ecological dominance of ants. Alternatively, perhaps they are ideal mutualistic partners because, compared with solitary living species, ant colonies are able to provide a more efficient, non-localised defence for vulnerable species. Not all ants are involved in mutualisms, however. By comparing the ecology of mutualistic versus non-mutualistic ant taxa, we are able to characterise ecological traits that are associated with mutualism. This allows insight into the origin of these mutualisms and can also lead to speculation over how mutualisms can shape species. In this study we focus primarily on the mutualism between ants and Homoptera (aphids, scales *etc*.). We consider how various ecological traits correlate with the occurrence of this mutualism in ant genera. In addition, we compare how the ant- Homoptera mutualism is associated with mutualisms between ants and other organisms.

Many ants are well known to tend Homoptera for their sugary 'honeydew'; a

process known as *trophobiosis*. In addition, ants also visit lycaenid butterfly larvae and the extrafloral nectaries of some plants to collect sugary secretions. These excretions/secretions contain a variety of amino acids and carbohydrates which ant colonies use to fuel foraging activity and feed developing brood. In return ants provide a wide range of benefits to their partners including protection from predators and parasites (Jones, 1929, Stadler & Dixon, 2005), shelter (Way, 1963), transport (Collins & Leather, 2002) and reduction of pathogen contamination (Fokkema et al., 1983, Way, 1954). The mutualism between ants and Homoptera is economically important because Homoptera are major agricultural pests worldwide and ants are ecologically dominant organisms having widespread impacts on the community structure (Wimp & Whitham, 2001) and nutrient cycling of ecosystems (Folgarait, 1998). There is broad diversity in the degree of association between aphids and ants. For example, one aphid species may often be tended by ants while a congener is not (Stadler & Dixon, 2005). Also, different aphids sharing the same host plant may differ in their degree of trophobiosis with ants (Bristow, 1991). A wide range of explanations have been suggested for the variation in trophobiosis in ant-aphid interactions; however, it is useful to distinguish between factors that promote variation in trophobiosis *within* species (i.e. across populations) and those that cause differences between species. Within a single aphid species there may be spatial and temporal variation in the degree of ant tending. Similarly, there will be differences between ant colonies in their investment in Homoptera. This variation across populations is characteristic of facultative mutualisms where both partners can survive alone and do not exclusively require interspecific association. Obligate mutualisms, in contrast, always find the two partner species in association. The majority of ant-aphid and ant-lycaenid relationships are thought to be facultative (Bristow, 1991, Pierce et al., 2002).

1   Population level variation in trophobiosis between ants and aphids can be

2   satisfactorily explained by two hypotheses. One is that, although relationships may be

3   mutually beneficial, ants and aphids are limited in their ability to encounter one

4   another. Ants are not omnipresent and their colony territories do not encompass the

5   entire landscape. Similarly, there is no evidence that alate aphid dispersal is directed to

6   patches with ants, even though apterous aphid dispersal may be limited by ant

7   semiochemicals (Oliver et al., 2007b). The second explanation is context dependent

8   fitness, whereby the costs and benefits of an interspecific association depend on a

9   multitude of biotic and abiotic factors which vary in time and space (Cushman &

10   Whitham, 1989). Thus, the net effect of an interaction on each partner may change in

11   magnitude and even sign (Bronstein, 1994, Thompson, 1994). For example, in cases

12   where costs exceed benefits, aphids will profit from leaving the association with ants.

13   This can be achieved through selection for traits that prevent ant tending e.g.

14   sequestering plant toxins in honeydew (Buckley, 1987). If the interaction frequently

15   fluctuates between mutualism and parasitism then natural selection can optimise

16   benefits by evolving phenotypically plastic traits that facilitate association only when

17   conditions are favourable e.g. increasing honeydew quality in the presence of ants (Yao

18   & Akimoto, 2002). A wide variety of ecological factors have been proposed to account

19   for population level variation in ant-aphid interactions including local species

20   composition and abundance of ants (Horvitz & Douglas, 1984, Oliver et al., 2007a) or

21   aphids (including competing mutualistic species) (Addicott, 1978); seasonal change in

22   ant nutritional requirements (Sudd & Sudd, 1983); presence of alternative sugar sources

23   (Offenberg, 2001) and host plant quality (Cushman, 1991, Auclair, 1963).

24   Variation in trophobiosis between ant and aphid *species*, however, can encompass

25   additional explanations. For example, morphological differences between ant species

1   in the development of the proventriculus can affect their ability to gather large

2   quantities of honeydew in the crop and thus affect suitability for trophobiosis

3   (Davidson et al., 2004). Factors, such as morphological change, operate on

4   evolutionary timescales and can shape species level variation. Evolution of third-party

5   antagonists can also disrupt mutualisms (Sachs & Simms, 2006). For example,

6   specialised parasitoids may arise which are able to subvert ant defences and because of

7   the enemy free space they find there, develop specially to exploit ant attended aphids

8   (Völkl 1992). Furthermore, mutualistic aphids may find themselves in competition with

9   other aphids which are attractive to ants (Addicott, 1978, Cushman & Addicott, 1989,

10   Cushman & Whitham, 1989). Dynamic indirect coevolution (mediated through the ant

11   mutualist) may ensue, in order to 'win' the attention of ants.

12   Generally, past attention has focused on aphids, with attempts to predict factors that

13   explain their asscociation with ants. Reduced alternative defences, such as cornicles

14   releasing waxy secretions and saltatorial legs for escape, have been hypothesised to

15   characterise ant attended aphid species, although evidence is equivocal (Bristow,

16   1991). Bottom-up effects of the type of host plant may go some way towards explaining

17   variation, with certain plant families (e.g Anacardaceae, 100%) supporting

18   considerably higher proportions of ant-tended aphids than others (e.g. Fagaceae, 2%)

19   (Bristow, 1991). Association with ants could also potentially be limited by aphid

20   phylogenetic contraints; however this does not appear to be the case (Bristow, 1991).

21   Indeed, there is evidence that association with ants is a highly labile trait that has

22   evolved and been lost multiple times in aphids (Shingleton & Stern, 2003). Feeding

23   location has been shown to predict some variation in ant attendance (Shingleton et al.,

24   2005). The most comprehensive analysis to date identifies feeding on woody plant

25   parts, non- mobile aphids, non- alate adults and aggregated colonies as traits that are

significantly associated with ant attendance (Stadler et al., 2003).

In comparison, there has been little attention given to factors that explain variation between ant species in their association with aphids. Indeed, it is not even known roughly what percentage of ants practice aphid tending. Only one study to date (ant proventriculus development (Davidson et al., 2004)), links an ant trait to interspecific association with trophobionts. The current study addresses this paucity of knowledge by identifying several ecological factors that are characteristic of homopteran-tending ants. We specifically test three *a priori* hypotheses, two of which have been suggested by previous researchers:

a) Ecologically dominant and polygynous ants are more likely to monopolise resources and thus form close associations with sugar-producing insects (Blüthgen et al., 2004, Fiedler, 1991, Eastwood & Fraser, 1999, Davidson, 1998).

b) Ant- Homoptera, ant- lycaenid and ant- extrafloral nectary plant mutualisms will be positively associated. Ant species that visit Homoptera and lycaenids appear to be a subset of those that visit extrafloral nectaries (Blüthgen et al., 2004, Fiedler, 2001). Liquid-feeding adaptations in ants may create opportunities to engage in all three mutualisms simultaneously.

c) Fungus gardening will be negatively correlated with trophobiosis because there is a trade-off preventing specialisation in two very different types of mutualism. In contrast, there will be a positive association with domatia- bearing plants as these have similar requirements to Homoptera (protection), yet provide different rewards.

Materials and methods

Data collection and analysis

Data on the ecological traits of 139 ant genera were collated from primary literature sources. The genera selected (139 of a possible 288 extant described genera (Moreau et al., 2006)) were those used by Moreau et al. (2006) in a large scale molecular phylogeny of the Formicidae. Binary ecological traits (see Table 1) were scored as positive if at least one species in the genus showed the trait. A similar approach has been used by Koh (2004) to model extinction risk in tropical butterflies. An obvious caveat with this approach is that, by chance, large genera are more likely to contain species with the trait. The sampling effort devoted to a genus may also have a similar effect, with the trait more likely to be found for well studied genera. To overcome this, we have included the log number of species in each genus and log sampling effort (number of hits on ISI Web of Knowledge) as explanatory variables to account for variation caused by these effects.

Most phylogenetic comparative analyses are usually conducted with species-level data, although genus-level data have also been used (Koh et al., 2004). In this case, the very large number of species in the Formicidae (~10,000) (Hölldobler & Wilson, 1990) and limited knowledge of many of these species makes any large scale analysis at the species level impossible. However, although there may exist polymorphism of a trait within a genus, such polymorphisms at the tips of a phylogenetic tree are a problem for any comparative analysis; species are assumed to have a certain trait yet in reality there may be intraspecific variation within the trait. Thus the question is not necessarily whether data is collected at genus level or species level, but rather are the proposed traits truly representative enough of the taxa? Although trait polymorphism within terminal taxa may present problems in the reconstruction of ancestral nodes and related

comparative methods that use such an approach e.g. phylogenetically independent

contrasts (Felsenstein, 1985), comparative methods that do not rely on ancestral state

reconstruction, but rather use a correlation matrix to weight non-independence, should

be suitable to model such traits. Despite this, for the majority of traits we have tested if

a trait is scored as a positive for a genera then it is likely that most species share the trait.

The two traits most likely to have higher degrees of polymorphism within genera are

polygyny and invasiveness.

To account for autocorrelation from phylogenetic non-independence we used

generalised estimating equations (GEE), which are similar to the GLS (generalised

least squares) approach (Grafen, 1989), except that non-normal errors in response

variables can be modelled (Paradis & Claude, 2002). They are suitable for data with

discrete response variables, through the specification of binomial, rather then Gaussian,

error structures, and also allow the exploration of interaction effects between

explanatory variables (Paradis, 2006, Paradis & Claude, 2002). We tested interaction

effects chosen only from *a priori* hypotheses (see Table 2).

For GEEs, a phylogeny is required to produce a correlation matrix derived from the

distances between terminal taxa in a tree, which is then used to weight variances in a

generalised modelling framework. We used a recent phylogeny of ants resolved to the

genus level by Moreau *et al.* (2006). GEEs were carried out using the 'compar.gee'

function in the 'ape' package (Paradis et al., 2004) of the program 'R 2.6.1' (Ihaka &

Gentleman, 1996, R.Development.Core.Team, 2007). The binary response variable

was the occurrence of homopteran tending (trophobiosis) in the genus and thus

binomial, rather than Gaussian, errors were specified with either logit or

complementary log-log links, depending on whichever minimised residual variance.

Computational iterations often diverged when both species count and sampling effort were included as blocking explanatory variables as these two are highly correlated (Pearson's product moment correlation: $t = 8.61$, d.f. $= 145$, $p < 0.001$). Therefore, only sampling effort was included. After testing each potential trait singly for an association with trophobiosis (Table 1), pairwise interactions were tested (Table 2), again with sampling effort as a blocking factor.

In addition to the GEE analysis, we also carried out an additional binary logistic regression analysis that does not account for relatedness between taxa. This analysis indicates association between ant traits, independent of shared evolutionary history between taxa, *i.e.* it simply asks: which traits best describe ant genera that tend aphids? If we then want to ask whether this association implies some kind of causal linkage between the traits rather than simply association through shared evolutionary history, we can then refer to the GEE analysis that takes phylogenetic non-independence of taxa into account. For the non-phylogenetic comparative analysis, binary logistic regressions specified with binomial errors were carried out with sampling effort and species count as additional explanatory variables.

Comparisons of the proportion of ant genera that are nomadic and/or tend Homoptera, and a similar comparison between dominance and trophobiosis were carried out using a binomial test calculating a Chi-squared statistic. The same test was used for the comparison between small and large dominant ants for the proportions of genera with certain other ecological traits (Table 5).

Results

Taxonomic distribution

Thirty seven percent of ant genera contained species that engage in trophobiosis with Homoptera. The taxonomic distribution was uneven with certain subfamilies more likely to contain mutualistic ant species (deviance = 52.195, d.f. = 18,119, p < 0.001) (Fig. 1). At the subfamily level there is a positive association between ant-trophobiont, ant fungi and ant-plant mutualisms (trophobiont-fungi: dev = 5.99, d.f. = 1,17, p = 0.014; trophobiont-plant: dev. = 4.11, d.f. = 1,17, p = 0.043; plant-fungi: dev. = 9.84, d.f. = 1,17, p = 0.002). Ant-fungi mutualisms always occur in subfamilies that also have mutualisms with plants and trophobiotic insects. For fungi, however, this positive association does not carry down to genus level and the trend becomes reversed (see next section). Over half of ant subfamilies do not engage in any known mutualisms with Homoptera, fungi or domatia bearing plants.

Ecological correlates of trophobiosis with Homoptera

Without controlling for phylogenetic non-independence there were a number of ecological traits significantly associated with trophobiosis (Table 3). Homopteran-tending ants are more likely to be dominant, polygynous, arboreal nesters with large colonies of 10^4-10^5 workers. Furthermore, invasive ants and ants that are found in disturbed habitats are more likely to be trophobiotic. There was a positive association between trophobiosis mutualisms and mutualisms between ants and domatia-bearing plants. Ninety four percent of the ant genera with species that live in plant domatia also contain trophobiotic species. In addition, ant-trophobiont and ant-plant mutualisms also co-occur at species level. For example most *Crematogaster* species that inhabit domatia of the ant-plant *Macaranga* keep scale insects (Homoptera) inside the plant chambers (Heckroth et al., 1998).

Using the GEE method to account for phylogenetic non-independence, many of these significant associations drop out (Table 4). This suggests a shared evolutionary history is responsible for the association in many of the cases. In contrast, those traits that are still significant after the GEE analysis are likely to have some causal relationship with trophobiosis because across the whole phylogeny they are repeatedly associated on many occasions. These associated traits include the other 'trophobiosis-like' mutualisms: feeding at extrafloral nectaries and the tending of lycaenid larvae, and also fungus gardening (a negative association). Six ant genera contain species that engage in symbiotic mutualism with fungi. Five of these six genera are attine ants (tribe Attini) that grow nutritional fungi in gardens and do not engage in trophobiotic mutualisms with Homoptera. The sixth genus, *Lasius* (subfamily Formicinae) is somewhat of an exception as only one species shares a mutualism with fungi. *Lasius fuliginosus* associates with an ascomycete fungi which grows in the walls of its carton nests and strengthens them structurally (Hölldobler & Wilson, 1990). Thus, in this trophobiotic genus there is an isolated occurrence of an ant-fungus mutualism, but it is not one in which ants derive nutrition from fungi.

There was a positive association between dominance of ant genera and trophobiosis (Table 4). Sixty seven percent of ant genera with ecologically dominant species contain homoptera-tending species, compared with only 23% of genera without ecological dominants. Interestingly, a significant association between nomadism and trophobiosis also emerged from the GEE analysis, whilst the effect was only close to significance in the binary logistic regression. Nomadic ants are less likely to tend Homoptera than non-nomadic genera. As with all comparative analyses, we can infer only association, not the direction of causality (nor whether it is direct or mediated through some other

1   trait); thus, whether these traits are a cause or consequence of trophobiosis is unknown.

2   In the absence of a species level phylogeny (onto which the origin of traits could be

3   more accurately mapped), one crude method to give a suggestion of the direction of

4   causality is to consider the proportions of genera (e.g. nomadic or trophobiotic) in each

5   group (e.g. trophobionts or nomadic ants, respectively). For example, if becoming

6   nomadic tends to cause ants to relinquish trophobiotic relationships then we would

7   expect the majority of nomadic ants to be non-trophobionts (lack of trophobiosis is

8   necessary for nomadism). In contrast, if not tending Homoptera caused ants to live a

9   nomadic lifestyle then we would expect most non-trophobiotic ants to become nomadic

10   (nomadism is necessary for non-trophobiosis). Considering the data, we find that a

11   significantly higher proportion of nomadic ants do not tend Homoptera (10/13, 77%),

12   compared with only a small minority of non-trophobiotic ants that happen to be

13   nomadic (10/84, 12%) ($\chi^2 = 25.2$, d.f. = 1, p < 0.001).This suggests that nomadism is a

14   precursor to the loss of the trophobiosis mutualism, and, thus, that relinquishing

15   trophobiosis is a consequence of being nomadic (but with many exceptions to this rule,

16   or reversions from trophobiosis after nomadism has developed, because only 77%

17   rather than a 100% of nomadic ant genera do not tend Homoptera). A similar

18   comparison for dominance and trophobiosis traits is less conclusive. Sixty two percent

19   of trophobiotic ant genera are dominant, compared with 76% of dominant ants that are

20   trophobiotic, the difference being non-significant ($\chi^2 = 1.69$, d.f. = 1, p = 0.193).

21

22   Finally, considering potential interaction effects between ecological factors (Table

23   2), there was a significant interaction between ecological dominance and mean ant

24   body size on the occurrence of trophobiosis with Homoptera (Binary log. reg.: Dev =

25   6.01, d.f. = 1,128, p = 0.014; GEE: t = 4.29, d.f. = 27.0, p < 0.001). Size had a

significant effect on the occurrence of trophobiosis only in dominant ant genera. The

direction of effect was opposite to that predicted, however: dominant ants tending

Homoptera are on average much smaller than dominants that do not (mean size

trophobionts($\pm$ SE) = 4.53$\pm$ 0.14mm, mean size non-trophobionts = 8.20 $\pm$ 1.40mm;

Dev = 6.213, d.f. = 1,35, p = 0.013). Thus dominant ants fall into two distinct groups,

those that tend Homoptera and those that do not, and several other ecological factors

also differ markedly between these two types of ant. Small, dominant ants that tend

Homoptera are more likely to be polygynous, invasive and thrive in disturbed habitats.

Furthermore, they are less likely to be nomadic or have polymorphic workers (Table 4).

There was no significant interaction between polygyny and colony size on

trophobiosis (Binary log. reg: Dev = 1.817, d.f. = 3, 69, p = 0.61; GEE: t =1.163, d.f. =

15.7, p = 0.284). Finally, consideration of an interaction effect between arboreal nesting

and latitude was not applicable as there were no arboreal nesting genera that occurred

only in temperate regions. For ants found solely in the tropics, 72 % of genera (18 out of

25) that tended Homoptera nested in trees. For ants found only in temperate zones, zero

out of nine genera that tended Homoptera nested in trees.

Phylogeny

The presence or absence of trophobiosis with Homoptera can be mapped onto a

phylogeny of the Formicidae (Fig. 2). With data collated at the genus level however,

ancestral state reconstructions can be inaccurate if there is considerable polymorphism

for the trait at the species level. Thus Figure 2 is an accurate reflection of the evolution

of trophobiosis given the assumption that, if trophobiosis is scored as positive for a

genus, then the majority of the species in the genus are trophobionts. If this assumption

is violated then there may be differences in the evolutionary history of trophobiosis

from what is shown here. For the trophobiosis trait, we believe that this assumption is reasonable. For example, in the recently resolved genus *Linepithema,* Homoptera tending is ubiquitous across all 19 species (Wild, 2007). Also, we have refrained from estimating the relative rates of transition between trophobiosis states, although it is reasonable to use the phylogeny to calculate the minimum number of state changes. Using the program PAUP (Swofford, 2000) the most parsimonious number of changes is an impressive 31. This figure is likely to be highly conservative as the phylogeny is resolved only to genus level and there may be many transitions at the species level. There may also be under-reporting in the literature which could lead to trophobiosis being incorrectly assumed to be absent in poorly sampled genera. This may slightly inflate or decrease the number of changes depending on whether it occurs in mutualistic or autonomous clades. Regardless, trophobiosis in ants appears to be a highly evolutionarily labile trait.

Discussion

The tending of homopteran and lycaenid insects by ants occurs on a broad taxonomic scale, with 45% of ant genera containing trophobiotic species. The taxonomic distribution is uneven, however, with certain subfamilies specialising in trophobiosis (e.g. the Aenictinae, Aneuritinae and Pseudomyrmicinae and of the larger subfamilies: the Dolichoderinae and Formicinae), while trophobiosis is entirely absent in others (e.g. the Ecitoninae). We have identified a number of ecological traits that are correlated with the occurrence of trophobiosis in ant genera. Thus, species most likely to tend Homoptera are ants that are polygynous, dominant in their community, arboreal nesting, with large colonies of $10^4 - 10^5$ individuals, and those that can occupy disturbed habitats. Several ecological factors, such as latitude and worker

polymorphism, did not correlate with trophobiosis. This could indicate these factors
have little influence on the evolution of trophobiosis. It is also possible however, that
they are important, but are simply not detected in a genus level analysis.

The association between dominance, polygyny and trophobiosis has been noted by
a number of researchers (Blüthgen et al., 2004, Fiedler, 1991, Eastwood & Fraser,
1999, Fiedler, 2001, Davidson, 1998). Dominant ant species can monopolise honeydew
producing resources by excluding subdominant species. Subdominant and specialised
ants tend to have smaller colonies and as such may be confined to peripheral ecological
niches where resources are more scarce. Thus a few key Homopteran species
colonising certain host plants can contribute to shaping the ant community of an
ecosystem (Blüthgen et al., 2004). At this point, however, we are unable to determine
which trait, dominance or trophobiosis, precedes which. Dominant ants may be more
able to outcompete others to obtain resources, but the converse is also possible whereby
the evolution of adaptations to exploit new resources allows ants to become dominant
(Davidson, 1998). Honeydew is a profitable, renewable source of carbohydrates as well
as essential amino acids (Völkl et al., 1999, Fischer & Shingleton, 2001). With access
to such food, ant colonies may be able to forage wider areas (Stadler & Dixon, 2005)
and support larger populations of vigorous workers able to outcompete other ants
(Davidson, 1997). Modification of the proventriculus is one such adaptation that
facilitates the trophobiont-ant relationship by allowing large amounts of liquid food to
be carried back to the nest. This may allow ants to increase numbers and achieve
dominance (Davidson et al., 2004). There may also be covariance in some of the
ecological traits important in trophobiosis. For example, polygynous ant colonies tend
to be large and polydomous (Hölldobler & Wilson, 1990); attributes which no doubt
help them to achieve ecological dominance and exclude competitors from sugar

1   producing resources (Davidson, 1998). Polygyny also breaks down within-colony

2   relatedness and so may also facilitate the formation of 'unicoloniality', very large

3   cooperative entities of ants that exclude competing species (Davidson, 1998).

4   Alternatively, Rosengren et al. (1993) have instead suggested that polygyny may result

5   *from* trophobiosis as the relative benefit of long distance dispersal by the female

6   reproductive class declines compared with remaining with the native colony in

7   competion-free, resource rich areas. Our study shows a positive association between

8   polygyny and trophobiosis with Homoptera, but this relationship disappears after

9   accounting for phylogenetic non-independence using the GEE method.

10   A novel finding in this paper is that dominant ants appear to fall into two categories:

11 small dominant ants that tend Homoptera and larger dominant ants that do not. A

12 number of other ecological traits also differ significantly between the two groups

13 (Table 5), supporting this grouping of dominant ants on the basis of homopteran

14 tending. Small ants may be more able to match worker allocation to resource value and

15 allow a continuous, steady flow of resources to the nest. In addition they may provide a

16 more effective, non-localised defence force through increased sensitivity (greater

17 number of- and more widely dispersed 'receptors'), followed by pheromone-mediated

18 mass recruitment towards targeted threats. Polygyny and polydomy can further aid this

19 resource monopolisation (Davidson, 1998) and invasiveness of ants may be an

20 emergent trait facilitated by a combination of small size, polygyny and homopteran

21 tending (Ness & Bronstein, 2004). Large dominant ants, in contrast, include ants such

22 as the Ponerines which tend adopt a more solitary mode of foraging. Also, on average,

23 large dominants have a greater degree of worker polymorphism which is also

24 associated with nomadic lifestyles.

25   It is noteworthy that trophobiosis was positively associated with invasiveness. This

could be due to traits that covary with trophobiosis, for example dominance.

Regardless, it highlights that the trophobiotic association with Homoptera is very

flexible. Invading ants can easily adopt new partners in a novel habitat. It may be that

general exaptations (preadaptations) of the ant-Homoptera mutualism allow easy

transfer to new partners (Mondor & Addicott, 2007) and a dynamic flux in mutualist

identity. Partner switching may occur not just with different congeneric species, but

across large taxonomic divides, such as the exploitation, by a single ant colony, of

many different Homoptera, lycaenid and extrafloral nectar-bearing plant species. This

may be especially useful if a partner's range and abundance is limited and also in novel

habitats where a previous partner is absent. Such partner switching may give facultative

mutualisms an advantage over obligate pairwise mutualisms. Although interactions

between ants and domatia-bearing plants (Beattie, 1985) or fungi (Mueller et al., 2005)

are often obligate and specialised, those between ants and sugar-producing

trophobionts are most often not (Pierce et al., 2002, Bristow, 1991). Rather than some

kind of clade selection promoting faculative mutualism over pairwise obligate

specialisation, however, in ant-Homoptera interactions we predict that facultative

mutualisms are more prevalent due to the dynamic metapopulation structure of

Homoptera combined with the by-product nature of rewards. Dynamic

metapopulations make reciprocal interactions between the same genotypes less

persistent thereby reducing partner fidelity. General by-product rewards (honeydew is a

waste product for Homoptera and ants, by nature, guard all types of food resource)

facilitate easy partner switching.

Finally, it may be intuitive to expect that close coevolution with a mutualistic

partner limits the extent to which a species can participate in other coevolutionary

relationships. Strong directional or balancing selection imposed by one coevolutionary partner may limit the degree to which a trait can be selected by another partner. We find, however, that trophobiotic ants are able to maintain several mutualisms simultaneously. Species that tend Homoptera are also highly likely to be those which tend lycaenids and feed at extrafloral nectaries. Ants provide similar benefits to each of these groups (primarily defence against enemies), and receive similar rewards (sugary secretions). Thus, general adaptations may facilitate all three mutualisms. For example, effective resource defence abilities, group recruitment and a modified proventriculus allow exploitation of these similar resources. In addition to maintaining mutualisms with trophobionts, ants can also simultaneously engage in mutualisms with domatia-bearing plants. A similar pattern of association has been found for plants with extrafloral nectaries: there is a positive association between extrafloral nectary bearing plants and ant-tended aphids (Offenberg, 2000). Ants that inhabit plant domatia often bring their Homopteran mutualists with them and use plant shelters to farm their herds. Similar to trophobionts, domatia-bearing plants require an effective defence force. By moving mutualistic partners together, ants can achieve this and reap the rewards from both (shelter from plants and food from Homoptera).

When requirements from mutualistic partners diverge, however, it appears ants may be required to specialise in one type of mutualism. Ants that garden fungi, for example, do not engage in trophobiotic interactions with Homoptera. Fungi require the gathering of growth substrates and maintenance of ideal conditions, Homoptera require a patrolling defence. Hoeksema *et al.* (2000) suggest that there may be allocation trade-offs in mutualisms, where limiting resources are allocated to traits associated with different partners. We suggest that foraging time and energy of ants may also be viewed as limiting resources, which trade off between potentially mutualistic partners such as

fungi and Homoptera. Over evolutionary time this partner choice becomes cemented by

the evolution of specialised traits that maximise benefits from a specific partner type,

but reduce the ability to exploit other partners. For example, each partner offers a

different type of food as a reward to ants, that may require different physiological

adapations to consume and digest. Where fungi can offer non-food rewards however,

(*e.g.* as a structural building tool in *L. fuliginosus* nests), then ants seem to be able to

adopt both Homoptera and fungi as mutualistic partners. Thus, from these observations

we propose as a general hypothesis that *species can specialise in multiple types of*

mutualism simultaneously only when there is no redundancy of rewards and no

trade-off in requirements (Fig. 3). In cases such as the ant mutualisms with Homoptera,

lycaenids and extrafloral nectaries there appears to be redundancy of rewards (all offer

sugary secretions) but no trade off in requirements (all require protection), a somewhat

'halfway house' satisfaction of the above criteria. In these cases we predict that the

species can maintain multiple *unspecialised faculative* mutualisms but specialised

obligate relationships will not develop. Conversely, for the other halfway house

possibility, when there is no redundancy of rewards but there is a trade-off in

requirements, we predict that there will be no multiple mutualisms maintained

whatsoever. Thus, the trade-off in requirements can be viewed as the most important

criterion in determining multiple mutualisms, with redundancy of rewards secondarily

determining whether the mutualisms are facultative or specialised obligate

relationships.

Conclusion

To summarise, we have identified a number of ecological traits that characterise

homopteran tending ants. Trophobiotic ants are more likely to be forest dwelling,

polygynous, ecologically dominant and arboreal nesting with large colonies of 10^4 –

1   10^5 individuals. Some of these associations remain even after accounting for

2   phylogenetic non-independence, suggesting causal relationships. For example, it is

3   highly likely that ecological dominance in ants is either responsible for- or a

4   consequence of- the maintenance of trophobiotic mutualism. We are not yet in a

5   position, however, to predict the direction of causality. We have also found that

6   dominant ants fall into two distinct groups depending on whether they tend Homoptera,

7   and these groups also differ in several related ecological traits, such as invasiveness,

8   polygyny and polymorphism. Finally, we observe that ants appear to specialise in

9   multiple mutualisms only when partners offer different types of reward and there is no

10   trade-off in requirements from the ants. We propose this as a general rule that may

11   govern the formation of multiple mutualisms by any species.

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14   Acknowledgements

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Tables

Ecological trait	Description	Factor type
Dominance	One or more species in the genus reported to have attained numerical dominance in their native community	Binary
Polygyny	One or more species reported as polygynous at least 6 months after colony founding	Binary
Polymorphic workers	One or more species have differentiated worker castes	Binary
Invasiveness	One or more species is an 'invasive' or 'tramp' species (McGlynn, 1999)	Binary
Arboreal nesting	One or more species of the genus nests in trees	Binary
Nomadism	One or more species have distinct nomadic phases with frequent daily colony movement	Binary
Trophobiosis with Lycaenidae	Species of ants in the genus also tend lycaenid caterpillars	Binary
Feed at extrafloral nectaries	Species of ants in the genus also feed at extrafloral nectaries	Binary
Latitude	The majority of species in the genus occur in temperate regions, tropical or both	Categorical
Colony size	Average colony size of the genus is small (0-200 workers), medium (200-10,000), large (10,000-100,000) or very large (100,000+)	Categorical
Body size	Mean body size of ants in the genus	Continuous
Habitat types	The number of different habitat types species of the genera occupy from a list of: forest, grassland, scrub, arid, wetland, disturbed	Continuous
Geographical range	The number of different biogeographic realms species of the genus occur in from a list of: Nearctic, Neotropic, Palearctic, Afrotropic, Oriental, Indomalay, Australasia	Continuous
Fungus gardening	One or more species grows fungus in gardens within the nest	Binary
Ant-plant mutualism	One or more species live in specialised plant domatia (Hölldobler and Wilson, 1990)	Binary
Blocking factor		
Subfamily	Subfamily of the genus (Moreau *et al.*, 2006)	Categorical
Species number	The number of species in the genus, obtained from the Species 2000 database	Continuous
Sampling effort	The number of hits for the genus on ISI Web of Science (ISI) search engine	Continuous

Table 1   Methods used for scoring ecological traits.

Interaction effect	Hypothesis
Arboreal nesting and latitude	Both temperate and tropical ants tend Homoptera, yet arboreal nesting species are generally found only in the tropics.
Polygyny and colony size	Polygyny may allow dispersed nests (polydomy), facilitating homopteran tending. Colony size is likely to covary with polygyny, but very small ant colonies are unlikely to be able to defend homopteran resources, even if polygynous.
Dominance and body size	Ants may need to attain a certain body size before becoming dominant enough to monopolise Homoptera.

Table 2 Hypotheses for pairwise interaction effects of ant ecological factors on the occurrence of trophobiosis with Homoptera

Ecological trait	Dev	df	P	Association	
Dominance	8.82	1,133	0.003	positive	**
Polygyny	7.02	1,93	0.008	positive	**
Polymorphic workers	1.01	1,114	0.315	none	
Invasiveness	4.28	1,136	0.039	positive	*
Arboreal nesting	10.34	1,130	0.001	positive	**
Nomadism	3.19	1,131	0.074	none	
Trophobiosis with Lycaenidae	85.22	2,135	<0.001	positive	***
Feeding at extrafloral nectaries	74.88	2,135	<0.001	positive	***
Latitude	3.02	2,134	0.221	none	
Colony size	4.84	1,92	0.027	large colonies	*
Body size	8.83	1,135	0.003	negative	
# Habitat types	4.92	1,131	0.027	positive	*
# Geographical Realms	0.14	1,135	0.709	none	
Fungus gardening	4.90	1,135	0.027	negative	*
Ant-plant mutualism	12.46	1,135	<0.001	positive	***
Habitat	**Dev**	**d.f.**	**P**	**Association**	
Forest	0.67	1,130	0.413	none	
Grassland	0.01	1,130	0.972	none	
Scrub	1.45	1,130	0.228	none	
Disturbed	5.45	1,130	0.020	positive	*
Wetland/ Heath	2.9	1,130	0.088	none	
Arid	0.56	1,130	0.453	none	

Table 3   Association between ecological traits or habitat types on the occurrence of trophobiosis with Homoptera.

The method involved binary logistic regressions with species number in genus and sampling effort as blocking factors.

Asterisks are significance codes for each ecological trait: *$0.01<p<0.05$, **$0.001<p<0.01$, ***$p<0.001$.

Ecological trait	t	df_P	P	Association	
Dominance	2.10	27.00	<0.001	positive	***
Polygyny	1.64	17.54	0.123	none	
Polymorphic workers	0.25	19.91	0.800	none	
Invasiveness	1.76	27.11	0.089	none	
Arboreal nesting	-	-	-	-	
Nomadism	2.11	21	0.049	negative	*
Trophobiosis with Lycaenidae	3.43	21.61	<0.001	positive	***
Feeding at extrafloral nectaries	3.57	21.61	<0.001	positive	***
Latitude	0.28	21.61	0.783	none	
Colony size	0.67	17.47	0.516	none	
Body size	0.04	20.84	0.967	none	
# Habitat types	0.58	22.12	0.56	none	
# Geographical Realms	1.73	21.61	0.101	none	
Fungus gardening	2.74	21.61	0.014	negative	*
Ant-plant mutualism	1.46	21.61	0.161	none	
Habitat	**t**	**dfP**	**P**	**Association**	
Forest	0.62	26.42	0.389	none	
Grassland	0.13	26.42	0.896	none	
Scrub	0.12	26.42	0.909	none	
Disturbed	1.35	26.42	0.191	none	
Wetland/ Heath	0.4	26.42	0.695	none	
Arid	0.91	26.42	0.373	none	

Table 4   Association between ecological traits or habitat types on the occurrence of trophobiosis with Homoptera, controlling for shared evolutionary history.

The method involved phylogenetic generalised estimating equations with sampling effort as a blocking factor. Phylogenetic degrees of freedom (df_P) are residual number of degrees of freedom corrected to account for branch length distances, to prevent inflated type I error rates (Paradis, 2006). Asterisks are significance codes for each ecological trait: *$0.01<p<0.05$, **$0.001<p<0.01$, ***$p<0.001$.

Characteristic	Small dominants	Large dominants	
Mean body size:	4.53mm	8.20mm	**
Tend Homoptera?:	Y	N	*
Polygynous	86%	36%	**
Invasive:	48%	0%	**
Disturbed habitats:	48%	12.5%	*
Polymorphic workers:	39%	80%	*
Nomadic:	6%	38%	*

Table 5 Characteristics of small dominant and large dominant ants.

Percentages given are of genera scoring positive for each trait. Asterisks are significance codes for each ecological

trait: *0.01<p<0.05, **0.001<p<0.01,***p<0.001.

Figures

Fig. 1 Ant subfamilies engaging in different mutualisms

The percentage of ant genera that contain mutualistic species in each subfamily. Subfamilies are in order of occurrence of trophobiosis with Homoptera, but also included are percentages engaging in mutualisms with plants and fungi.

Fig. 2 Cladogram of the Formicidae showing trophobiosis with Homoptera

- please see attached PDF.

Fig. 3 Multiple mutualisms cannot occur if there is a trade-off in requirements and redundancy of rewards

Ants simultaneously maintain specialised mutualisms with Homoptera (A) and domatia bearing plants (B). In contrast, there is a negative correlation between ant- Homoptera (A) and ant –fungi (C) mutualisms.
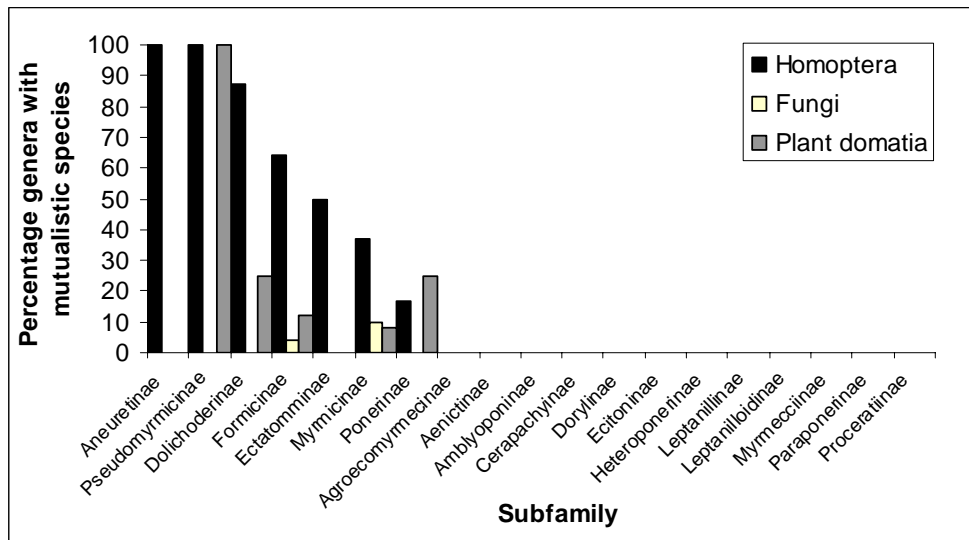
Figure Legends

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Fig. 2 Cladogram of the Formicidae showing trophobiosis with Homoptera

Trophobiosis occuring within a genus is shown in black. The six bottom-most genera are vespoid and sphecoid outgroups.
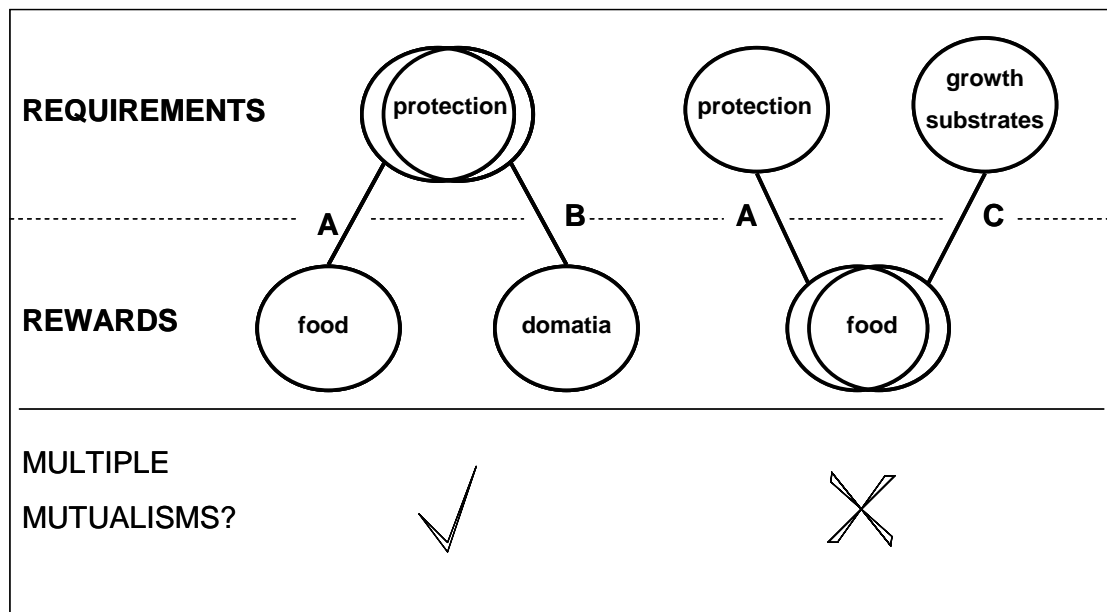
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Table Legends

Table 1 Methods used for scoring ecological traits

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Table 4 Association between ecological traits or habitat types on the occurrence of trophobiosis with Homoptera, controlling for shared evolutionary history.

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Percentages given are of genera scoring positive for each trait. Asterisks are significance codes for each ecological trait: *$0.01<p<0.05$, **$0.001<p<0.01$,***$p<0.001$.

Eutetramorium
Colobostruma
Rostromyrmex
Wasmannia
Wyrmicaria
Wyrmica
Megalomyrmex
Cetramorium
Calyptomyrmex
Crematogaster
Melissotarsus
Rhopalomastix
Xenomyrmex
Diploata
Diplorhoptrum
Dolichodorax
Leptothorax
Temnothorax
Mayriella
Solenopsis
Cardiocondyla
Monomorium
Oligomyrmex
Carebara
Pheidologeton
Atopomyrmex
Meranoplus
Rhoptromyrmex
Podomyrma
Metapone
Aphaenogaster
Goniomma
Oxyopomyrmex
Stenamma
Messor
Pogonomyrmex
Ocymyrmex
Myrmica
Manica
Spurogenys
Eurhopalothrix
Cyphomyrmex
Atta
Trachymyrmex
Sericomyrmex
Cephalotes
Procryptocerus
Pheidole
Cranopelta
Cataulacus
Protomonica
Romerjyra
Oecophylla
Stigmacros
Opisthopsis
Polyrhachis
Camponotus
Anoplolepis
Cecropia
Eupronolepis
Myrmecorhynchus
Melophorus
Prolasius
Notostigma
Plagiolepis
Prenolepis
Pseudolasius
Xyrmecocystus
Acanthomyops
Lasius
Myrmelachista
Brachymyrmex
Hylidoponera
Cctatomma
Gnamptogenys
Heteroponera
Boltrioponera
Dolichoderus
Tapinus
Bothriomyrmex
Technomyrma
Phillidrelus
Iridomyrmex
Froggattella
Ponyrmyrmex
Azteca
Leptomyrmex
Technomyrmex
Pseudomyrmex
Tapinoma
Sphinctomyrmex
Tetraponera
Myrmecia
Aenictus
Dorylus
Dorymyrmex
Ecion
Labidus
Neivamyrmex
Nomamyrmex
Sphinctomyrmex
Cerapachys
Cerapachys
Acanthostichus
Platythyrea
Cryptopone
Brachyponera
Pachycondyla
Diacamma
Hypoponera
Myopone
Streblognathus
Odontomachus
Anochetus
Odontoponera
Centromyrmex
Paturidris
Plagiolepis
Apomyrma
Prionopelta
Concoctio
Onychomyrmex
Amblyopone
Adetomyrma
Mystrium
Discothyrea
Proceratium
Probolomyrmex
Protanilla
Leptanilla
Phyllopes
Evageles
Odontophotopsis
Sapyga
Philanthus
Apis

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