# Public goods, public services and by-product mutualism in an ant–fern symbiosis

**Tom M. Fayle, David P. Edwards, Edgar C. Turner, Alex J. Dumbrell, Paul Eggleton and William A. Foster**

*T. M. Fayle (t.fayle@imperial.ac.uk) and E. C. Turner, Forest Ecology and Conservation Group, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot, Berkshire, SL5 7PY, UK. TMF also at: Insect Ecology Group, Univ. Museum of Zoology Cambridge, Downing Street, Cambridge, CB2 3EJ, UK. – P. Eggleton: Entomology Dept, Natural History Museum, Cromwell Road, London SW6 5BD, UK. ECT and W. A. Foster: Insect Ecology Group, Univ. Museum of Zoology Cambridge, Downing Street, Cambridge CB2 3EJ, UK. – D. P. Edwards, Woodrow Wilson School of Public and International Affairs, Princeton Univ., Princeton, NJ 08544, USA. – A. J. Dumbrell, Dept of Biological Sciences, Univ. of Essex, Wivenhoe Park, Colchester, CO4 3SQ, UK.*

Mutualism can evolve when organisms make novel connections that happen to benefit all parties. When such connections involve a host that provides a public good for multiple visitor species, selection for reciprocal cooperation is only likely if the host has the power to preferentially increase the fitness of those visitors that provide a better quality service. In contrast, when interactions form between multiple visitors and a host that lacks the power to partition the public good, we predict that interactions are likely to persist only as by-product mutualisms, in which symbiosis benefits both host and visitor, but in which neither partner makes costly investments. Focusing on the symbiosis between ants and litter trapping epiphytes in the canopy of lowland dipterocarp rain forest in Borneo, we show that at least 71 ant species share the public good of housing within the root-mass of epiphytic bird's nest ferns (*Asplenium* spp.). Ferns supporting a higher biomass of ants experienced less herbivory, and experimental exclusions of ants from fronds confirmed that this is caused by the ants protecting the ferns from herbivores. These results establish that there are clear by-product benefits for both parties of housing for ants and protection for ferns. If these benefits were to drive selection for reciprocal cooperation, we would expect larger ferns to support ant colonies that were larger or colonies that produce more reproductive individuals. This was not the case. Larger ferns instead supported more ant colonies, indicating that the housing provided by the fern is a public good that is not restricted to better cooperating ant species. Mutualism via novel connections is thus unlikely to evolve between a host and multiple partners, even if there are clear by-product benefits to all parties, unless the host can direct benefits to cooperators.

A central aim in the study of mutualism is to understand the mechanisms that promote the evolution of cooperation. Many symbioses apparently evolve from interactions that are initially antagonistic (Gibson and Hunter 2010), via by-product benefits (Leimar and Connor 2003), the evolution of tolerance mechanisms (De Mazancourt et al. 2005, Edwards 2009), or sensory traps (Edwards and Yu 2007), which enable species to obtain a greater benefit from cooperation than from antagonism. A second important source of mutualistic interactions is likely to be 'partner capture', in which free-living organisms make connections de novo or via symbiont switches to new hosts (Douglas 2010). Partner capture differs from the evolution of mutualism via antagonistic relationships, because novel interactions immediately yield beneficial outcomes for both parties.

The evolution of mutualism following partner capture is likely to require a partner choice or partner fidelity feedback mechanism to further align the interests of partners (Sachs et al. 2004, Foster and Wenseleers 2006, Weyl et al. 2010). Partner choice mechanisms permit a host to selectively associate with a particular cooperating genotype and

to avoid association with less beneficial or cheating genotypes. For instance, in the ant–*Leonardoxa* plant interaction, where plants trade housing for protection from herbivores, each *Leonardoxa* species has evolved a specialised domatia (ant housing) entrance. The unique shape of each entrance hole matches the head shape of the symbiotic queen, permitting colonisation, but filters queens of cheater ant species, which have different head shapes (Brouat et al. 2001). In the context of partner capture, if a novel beneficial species joins a multi-species pool of potential symbionts (Kiers et al. 2003, Archetti et al. 2011), then partner choice could promote association with this new species over less beneficial or cheating genotypes.

Once associations are made, power asymmetries in newly formed relationships can enable the partner holding the physical resource (typically the host) to coerce the partner providing the service (the visitor) into acting cooperatively by withholding rewards from non-cooperators and/or directing benefits to better service providers (West et al. 2002, Kiers et al. 2003, Edwards et al. 2006, Archetti et al. 2011, but see Weyl et al. 2010). Most instances of host coercion

of symbionts are partner fidelity feedbacks, in which the host responds to each outcome (or 'signal') in the best way possible for that outcome, without any inference of symbiont behaviour (or 'action'; Weyl et al. 2010). Partner fidelity feedbacks are based upon preadapted traits (Weyl et al. 2010, but see 'host sanctions' therein), and host responses are thus the same regardless of whether a particular outcome is the result of visitor action or of stochastic environmental events. An example is the *Allomerus* ant–*Cordia nodosa* plant interaction, in which the growth of new domatia is tied to the successful development of the attached new leaves. This selects for ant investment in new leaf protection, because the reproductive output of *Allomerus* is strongly positively related to the number of domatia on a plant (Edwards et al. 2006, 2007).

Partner capture can involve multiple visitor species that receive a public good from their host and that in return provide a public service to their host (Kiers et al. 2003, Archetti et al. 2011). In such cases, selection for reciprocal investment, and thus the evolution of mutualism, is only likely if a partner fidelity feedback mechanism exists for benefits to be directed to more cooperating individuals (Archetti et al. 2011). However, if the host lacks such a mechanism (Edwards et al. 2010), non-cooperating visitors could exploit the public good provided by their neighbours, reducing benefits for cooperators and potentially shifting the cost-benefit ratio such that defection as a cheater is the best strategy for cooperators. As a result, in instances when cooperative interactions form between multiple visitors and a host that lacks a method of directing benefits to better performing partners, we predict that interactions are likely to persist only as by-product mutualisms. In such relationships, neither partner is likely to make costly investments and instead reciprocal benefits are by-products of behaviours adapted for other purposes.

Here, we focus on the symbiotic interaction between ants and epiphytic host plants, which has repeatedly resulted in the evolution of mutualism (Huxley 1980). In many of these systems partners invest in their cooperative relationships: ants receive housing within preformed plant cavities and/or are provided with food, and in return, ants provide plants with nutrients or actively patrol for herbivores (Huxley 1980, Treseder et al. 1995). Such relationships usually involve a single ant colony per host plant, and thus private goods and services are traded directly between the host and ant colony. However, some epiphytes are colonized by multiple ant colonies, thus supporting a range of species at any one time (Ellwood et al. 2002, see also Gibernau et al. 2007), all of which share the public good of housing. We do not yet know whether epiphytes benefit from housing multiple species and whether any public services provided by the ants result in benefits for better co-operators, which would drive the evolution of mutualism.

Here we quantify public goods and public services for epiphytic bird's nest ferns (*Asplenium* spp.) and their ant symbionts (Fig. 1). Bird's nest ferns are very abundant, being found at densities of 180 plants ha$^{-1}$ in this study. They intercept falling leaf litter in their fronds, which decomposes, percolating down through the fern core, and probably provides the ferns with the majority of their required nutrients (Turner et al. 2007). Within this litter-mass lives a diverse arthropod community, a large proportion of which are ants (Ellwood et al. 2002, Turner and Foster 2009, Fayle et al. 2010). However, because ants nest in the litter-mass suspended within the fern core rather than in modular plant grown structures (Brouat et al. 2001), we expect that colonisation by ants is not under the control of ferns (and thus that there is no partner choice). Furthermore, since ants inhabit an undifferentiated mass of decomposing litter, we also expect that ferns would be unable to direct benefits to



Figure 1. (A) Bird's nest fern (*Asplenium phyllitidis*) in the rain forest understory. Note the intercepted leaf litter and sponge-like core. For scale, diameter of supporting tree = 4 cm. (B) Colonies of ants such as these *Diacamma rugosum* create nests by hollowing out cavities in the fern core. Horizontal width of cavity = 3 cm.

cooperators or costs to cheaters, via partner fidelity feedback, in this system. We therefore predict that partner capture will result at most in by-product benefits to multiple ant visitors and non-discriminating ferns. In this study we use a combination of observations and experiments to 1) quantify partner capture by *Asplenium* spp., 2) determine whether multiple ant-symbionts provide the public service of protection from herbivores, and 3) determine whether ant visitors gain partner fidelity feedback benefits of frequenting larger host ferns.

## Methods

### Ant–fern censuses

Eighty-three *Asplenium* epiphytic ferns and their ant inhabitants were collected from lowland dipterocarp rain forest near Danum Valley Field Centre (DVFC), Sabah, Malaysia (5°01′N, 117°49′E) in April–July 2006 (see Fayle et al. 2009 for further site details). Ferns were identified to species (*Asplenium nidus* and *Asplenium phyllitidis*), and at the time of collection, we measured a number of variables: canopy cover, substrate diameter, canopy openness, height in canopy and fern size (see Supplementary material Appendix 1 Table A1 for measurement details).

Ferns were dissected using a fine-toothed saw and secateurs, and fern material was placed in Winkler extractors for three days, following Fayle et al. (2010). Fern material was then processed by hand to remove any remaining ants, and dried to constant weight. All ants were identified to genus using published keys (Supplementary material Appendix 1), classified into morphospecies, and where possible, assigned to named species. Founding queens that did not have any associated workers were not included in analyses, nor were strays of large-bodied species that were deemed unlikely to have colony centres within the fern. Ant biomass was calculated using standard regression equations linking mass with body length (Schoener 1980).

### Quantifying ant partner capture by host ferns

We used ordination techniques to assess the difference in ant communities between the two fern species while taking into account the effects of environmental factors on ant species composition. Presence–absence data were used, since ants are colonial. Ant species that only occurred once in the dataset and ferns with 0–1 colonies were excluded from analysis. Because the maximum gradient length for an initial unconstrained ordination (DCA) was greater than four, indicating high levels of species turnover, we chose a constrained ordination method (Canonical correspondence analysis (CCA)) that assumes a unimodal response to underlying environmental gradients.

To test whether or not partner capture by ferns occurred at random or was restricted to a subset of ant species, we used existing data (Fayle et al. 2010) to compare the ant communities inhabiting *Asplenium* ferns with those from the leaf-litter of the forest floor below, which offers a largely similar microhabitat to fern leaf-litter. We used ordination analyses (DCA, CCA) to test whether or not the ant communities inhabiting the leaf-litter of 20 bird's nest ferns differed from those communities found in the leaf-litter of 20 paired samples taken from the forest floor below each fern. To estimate the degree of overlap between ground-litter and fern-litter ant communities we used an incidence-based estimator of species overlap that takes into account undersampling of communities (Chao et al. 2005, Colwell 2009).

### Does inhabitation by ants reduce levels of herbivory?

Since bird's nest ferns probably obtain substantial amounts of nutrients from trapped leaf litter (Turner et al. 2007) they are unlikely to require feeding by ants. Consequently, the most likely service provided by their ant inhabitants is protection from herbivores. We assessed herbivory rates for all 83 ferns sampled by counting the total number of fronds on each fern and scoring each frond as either 1) showing signs of herbivory or 2) not damaged by herbivory. Using generalized linear models (GLzMs) with binomial error and a logit link (glm function in R package base), we analyzed the effects of ant community structure (ant abundance, ant colony biomass and number of colonies) on herbivory rates. We included all environmental and fern characteristics as covariates in initial models (Supplementary material Appendix 1 Table A1). Continuous explanatory variables were $\log_{10}(x + 1)$ transformed, canopy cover was arcsine($\sqrt{p}$) transformed. In a second analysis, we assessed the relationship between the presence of individual ant species (present in ≥ 10 ferns) and herbivory rates, with the presence/absence of a particular species being included as a binary factor. Finally, to assess whether any overall relationships between community composition and herbivory rates were driven by individual species, we reran the first set of analyses excluding any ant species that were found to affect herbivory rates. We generated partial regression plots for independent variables of interest for these analyses. Residuals were plotted for each model (glm.diag.plots in R package boot) and datapoints with disproportionate influence were removed (three data-points for most models).

We then conducted experimental exclusions of ants from fern fronds to test whether any relationship between ant inhabitation and was causal. Forty intermediate-sized ferns (50–180 cm maximum diameter frond tip to tip) were selected between heights of 0.8 m and 3.3 m at random from a known population of ferns, surveyed as part previous studies (Turner et al. 2007, Turner and Foster 2009). Two fronds of a similar size and with minimal evidence of herbivore activity were selected on each plant: these two fronds were randomly assigned to experimental and control treatments. The base of the stipe of each experimental frond was covered with tanglefoot to prevent access by patrolling ants, while controls were left untreated. Care was taken to ensure the leaf was otherwise isolated from the rest of the fern. Although many herbivores are able to fly, the exclusion of non-flying herbivores from our treatment fronds (because they are unable to access the frond at the stipe) means that our exclusion experiment represents a conservative estimate as to the intensity of any protection received by ferns from their ant symbionts. Herbivory was measured after two months by cutting both the control and experimental fronds from the plants and measuring the total area of each frond

and the area of the frond removed by herbivores. The proportion of frond area removed by herbivores was compared between paired control and exclusion treatments using beta regression with a logit link function (function betareg in the R package *betareg*, Cribari-Neto and Zeileis 2010).

### Do protecting ants receive partner fidelity feedbacks?

In order for partner fidelity feedbacks to occur and thus for mutualism to evolve beyond the provision of only by-product benefits, individual ant species must benefit from any direct investment in fern fitness and the resulting increase in fern size. Since plant-ants are nest site limited (Fonseca 1993, Yu et al. 2004, Edwards et al. 2010), such partner fidelity feedbacks are likely to result from the provision of new nesting space as ferns grow, provided that resident ant colonies can exploit the new nesting space. We therefore tested whether larger ferns supported 1) larger colonies of particular species (those present in ≥ 10 ferns) and/or 2) more ant colonies, by using general linear models (GLMs) to assess the effect of fern size and environmental variables on the number of ant colonies, total ant biomass, and total ant abundance. To investigate any reproductive benefits of inhabiting a larger fern, we also analyzed the relationship between the number of winged reproductive individuals (male and female) in a colony and fern size. These analyses were restricted to the two ant species that had ≥ 5 colonies with reproductive individuals. For both univariate and multivariate analyses the least significant variables were removed in a stepwise

manner from an initial full model to obtain the model with the lowest AIC (Akaike information criterion, Maindonald and Braun 2007, p. 194).

## Results

### Quantifying ant partner capture by host ferns

An extraordinarily diverse community of ants frequented our 83 study *Asplenium* ferns, with 71 ant species in 27 genera having resident colonies (Table 1). This is a higher diversity than many other ant–epiphytic plant systems (Supplementary material Appendix 1 Fig. A1). Individual ferns were frequently occupied by ants with 89% (n = 74) having at least one ant colony and 59% (n = 45) having multiple colonies (mean ant colonies per fern = 2.8 ± 0.3 SE, range 0–12), indicating that housing within *Asplenium* spp. is a public good. No single species was widespread, with the two commonest resident ant species (*Hypoponera* #039 and *Monomorium* #009) each occurring in only 18% of ferns (n = 15). Nevertheless, occupancy rates were highly variable between species (Table 1), indicating that some ant species maintained more frequent interactions with host *Asplenium* than other inhabitants.

Ant species composition differed between ferns of different sizes and also between ferns at different heights in the canopy (Supplementary material Appendix 1 Table A2, Fig. A2). However, after controlling for these variables, there was no difference in composition between the two species

Table 1. Summary of the fern ant fauna showing the numbers of subfamilies, genera and number of species within each genus occurring in 83 ferns. Where it was possible to assign species names to morphospecies these are given (morphospecies ID numbers are not presented). Also presented is the total number of colonies of each genus found and the mean and range of colony size for each genus. Ant identification references are given in Supplementary material Appendix 1.

| Subfamily | Genus | Species | No. species | Colonies | Colony size mean (range) |
|---|---|---|---|---|---|
| Dolichoderinae | *Tapinoma* | | 1 | 2 | 9 (6–11) |
| | *Technomyrmex* | | 1 | 2 | 52 (49–55) |
| Ectatomminae | *Gnamptogenys* | *menadensis* | 1 | 1 | 136 |
| Formicinae | *Camponotus* | *gigas* | 6 | 12 | 100 (7–317) |
| | *Paratrechina* | | 6 | 18 | 47 (3–207) |
| | *Polyrhachis* | *noesaensis, equina* | 4 | 4 | 155 (2–391) |
| Ponerinae | *Anochetus* | *myops* | 2 | 2 | 19 (2–37) |
| | *Cryptopone* | *testacea* | 1 | 2 | 3 (2–4) |
| | *Diacamma* | *rugosum* | 2 | 11 | 34 (15–63) |
| | *Hypoponera* | | 3 | 19 | 25 (1–143) |
| | *Leptogenys* | *diminuta* | 4 | 6 | 32 (9–108) |
| | *Odontomachus* | | 1 | 1 | 399 |
| | *Pachycondyla* | *tridentata* | 2 | 14 | 21 (2–50) |
| | *Ponera* | | 3 | 19 | 5 (1–23) |
| Proceratiinae | *Discothyrea* | | 1 | 1 | 9 |
| | *Proceratium* | | 1 | 1 | 8 |
| Myrmicinae | *Cardiocondyla* | | 1 | 4 | 42 (3–86) |
| | *Carebara* | | 6 | 23 | 46 (1–268) |
| | *Crematogaster* | *modiglianii* | 4 | 12 | 1336 (35–5667) |
| | *Monomorium* | | 1 | 15 | 259 (2–1859) |
| | *Pheidole* | | 5 | 13 | 272 (17–965) |
| | *Pheidologeton* | *pygmaeus* | 1 | 1 | 1583 |
| | *Pyramica* | *mitis* | 4 | 20 | 19 (1–324) |
| | *Rhopalomastix* | | 2 | 2 | 10 (6–14) |
| | *Solenopsis* | | 1 | 4 | 233 (41–429) |
| | *Strumigenys* | *godeffroyi, treptodens* | 6 | 26 | 10 (1–72) |
| | *Tetramorium* | *ocothrum* | 1 | 2 | 116 (103–129) |

of fern. The species composition of ant communities found in the ferns differed from those inhabiting litter on the forest floor (CCA, Monte Carlo permutation test: $F = 2.78$, nperm = 999, $p = 0.001$). Estimators of species overlap indicated that although only 18% of the species from the forest floor are able to colonise the ferns (59.5 of an estimated total of 318.4 species), these species represent the majority of ants found in the ferns, with 83% of fern dwelling ants also inhabiting the leaf litter (59.5 of an estimated total of 70.9 species). Consequently, there is clear evidence of non-random partner capture of multiple ant species, but there is no evidence for differential partner choice between fern species, nor any evidence that one ant species is an obligate inhabitant of either species of fern.

## Does inhabitation by ants reduce levels of herbivory?

Ferns with a higher biomass of resident ants had a smaller proportion of their fronds attacked by herbivores (GLzM: $z = -3.81$, $p < 0.001$, Fig. 2A). Larger ferns, those ferns with lower canopy openness, and those of the species *A. nidus* also experienced less herbivory (GLzM: fern size: $z = 9.95$, $p < 0.001$; canopy openness: $z = -3.12$, $p = 0.002$; fern species: $z = -4.36$, $p < 0.001$). This excludes the possibility that the effect of ant biomass on fern herbivory is due to a confounding correlation with fern size. All other predictor variables, including ant abundance and species richness, were removed from the final model (all $p > 0.30$). Of the five commonest ant species, only *Monomorium* #009 affected rates of herbivory: ferns supporting this species experienced less herbivory than those without it (Fig. 2B,

GLzM: $z = -4.57$, $p < 0.001$; other ant species $p > 0.10$). However, the overall pattern of reduced herbivory with more ant biomass (Fig. 2A) persisted even when *Monomorium* #009 were excluded from the analysis (GLzM, effect of ant biomass on proportion of fronds with herbivory: $z = -3.84$, $p < 0.001$), indicating that the public service of protection is not driven by a single cooperating species.

Experimental exclusion of ants from individual fronds resulted in an increase in the proportion consumed by herbivores compared to controls with patrolling ants (Fig. 2C, Beta regression: $z = 2.97$, $n = 40$ pairs, $p = 0.003$).

## Do protecting ants receive partner fidelity feedbacks?

Both the number of colonies and ant biomass were related to the size of the fern and to no other environmental variables (Fig. 3A–B, Supplementary material Appendix 1 Table A3, no. of colonies: $t_{1,81} = 8.00$, $p < 0.001$; ant biomass: $t_{1,81} = 8.75$, $p < 0.001$). Higher abundances of ants were found in larger ferns (Supplementary material Appendix 1 Table A3, fern size: $t_{1,80} = 8.10$, $p < 0.001$) and in those of the species *A. phyllitidis* rather than *A. nidus* (Fig. 3C, Supplementary material Appendix 1 Table A3, species: $t_{1,80} = 3.00$, $p = 0.004$). For the five commonest ant species there was, however, no relationship between colony size and the size of the inhabited fern (*Pachycondyla tridentata* $F_{1,8} = 0.88$, $p = 0.37$; *Carebara* #020: $F_{1,12} = 0.17$, $p = 0.69$; *Pyramica* #018: $F_{1,11} = 0.27$, $p = 0.61$; *Hypoponera* #039: $F_{1,13} = 2.30$, $p = 0.153$; *Monomorium* #009: $F_{1,13} = 0.50$, $p = 0.49$; Supplementary material Appendix 1 Fig. A3). Furthermore,
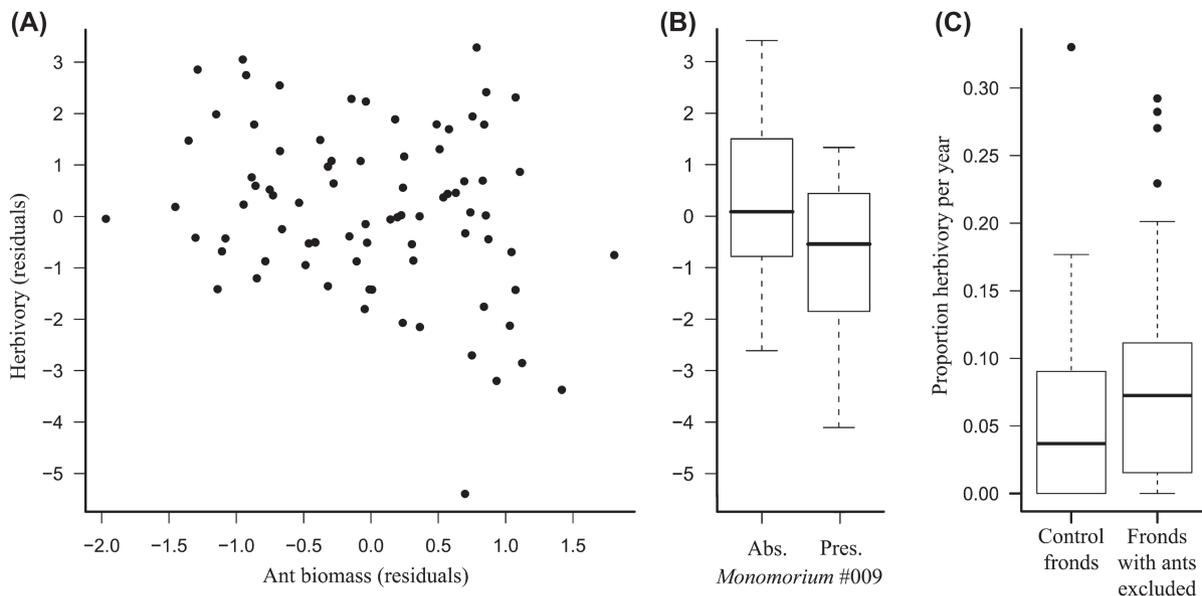


Figure 2. (A) Ferns with a higher biomass of resident ants experienced less herbivory. For example, a 144 g *A. phyllitidis* fern in an area with average canopy openness is predicted to have 87% of its fronds showing signs of herbivory when uninhabited, but only 68% when inhabited by a total of 2000 mg of ants (this is approximately the range of real ant biomass values for ferns of this size, Fig. 3). (B) The presence of *Monomorium* #009 led to reduced levels of herbivory. However, note that the overall relationship between rates of herbivory and total ant biomass remains even when this species is excluded from analysis. Plots (A) and (B) are generated using residuals from a partial regression analysis for the variable of interest, in which the x-values are the residuals from a regression of all other independent variables against ant biomass and the y-values residuals from a regression of all independent variables apart from ant biomass against herbivory rates. (C) Exclusion of ants from fronds resulted in an increase in the frond area consumed by herbivores relative to control fronds. Note that the experimental exclusion and control fronds were paired.
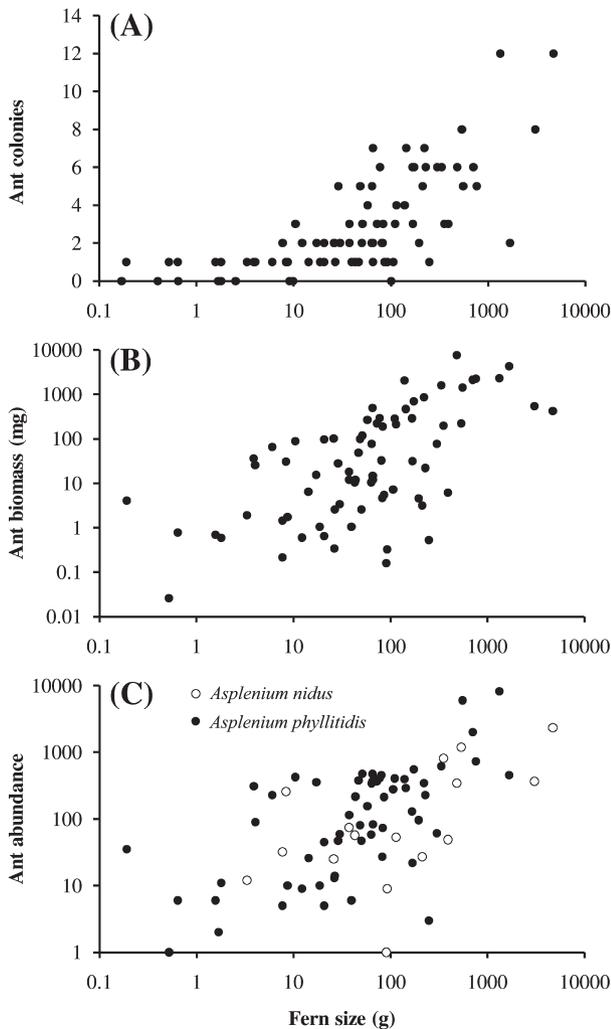
Figure 3. (A) Larger ferns supported more resident ant colonies (GLM: t = 8.00, n = 83, p < 0.001). Note the logarithmic scale of the x-axis. (B) Larger ferns also supported a greater total biomass of resident ant colonies (GLM: t = 8.76, n = 83, p < 0.001). Note the logarithmic scale on both axes. (C) Larger ferns supported more ants (GLM: t = 8.10, n = 83, p < 0.001). In addition, for a given size of fern, ferns belonging to the species *Asplenium phyllitidis* (filled points) supported more ants compared to *Asplenium nidus* ferns (open points) (GLM: t = 3.00, n = 83, p = 0.004). However, larger ferns did not support larger colonies of individual ant species, or colonies with more reproductive individuals (Fig. A3). For statistics relating to non-significant predictors see Table A3.

there was no relationship between fern size and abundance of reproductives (*Hypoponera* #039: $F_{1,13} = 0.17$, p = 0.68; *Monomorium* #009: $F_{1,13} = 2.60$, p = 0.13). Consequently, there is a common interest in inhabiting a larger fern, but no potential fidelity feedback benefit at the individual species level.

## Discussion

*Asplenium* ferns were commonly inhabited by ants, with rates of occupancy comparable to or higher than that found in ant–plant systems where housing is traded for protection (Yu and Davidson 1997, Heil et al. 2009, Edwards et al.

2010). Some host-ant species were more widespread inhabitants, indicating that they may maintain regular interactions with *Asplenium* whilst others appeared to be more transient visitors (Table 1). Furthermore, since the species composition of ant communities in the ferns differed from those found on the forest floor, while still remaining a nested subset of that community, partner capture is non-random.

Partner capture probably results from the favourable microhabitat within fern root/litter-mass, which is less variable than the climatic extremes found in the surrounding canopy (Turner and Foster 2006). Perhaps owing to the frequent capture of ant partners, the two species of fern did not support differing suites of ant species and there was still a large overlap in species between fern and forest floor, indicating that ant–fern relationships are not obligate. Nevertheless, our results do indicate that *Asplenium* provide an extraordinarily diverse community of ants with the by-product benefit of housing and that housing represents a public good, because many ferns are inhabited by more than one colony at a time. The provision of a public good here may have effects that scale up to the level of ant communities in the canopy as a whole, since nest site availability is thought to strongly limit these ants (Philpott and Foster 2005).

Inhabitation by ants resulted in potential fitness benefits for ferns, with ferns inhabited by a greater biomass of ants suffering less herbivory (Fig. 2A) and with the exclusion of ants leading to increased levels of herbivory compared to ant-patrolled fronds (Fig. 2C). We therefore show that entire ant communities provide their ferns with the public service of protection. The presence of *Monomorium* #009 also decreased herbivory levels compared to ferns that lacked this ant species (Fig. 2B), suggesting that different ant species vary in their provision of protective services to ferns and that there is the potential for the evolution of more cooperative ant species in this system (see also Edwards et al. 2010, Heil et al. 2009 for recent examples of variation in cooperation in ant–plant symbioses). Intriguingly, variation in the quality of service provision is the expected result of a non-linear public goods game, in which the beneficial effect of ants investing in protection is saturating. In such situations the equilibrium outcome is a mixed strategy with some players protecting and some not protecting, with the number of co-operators decreasing in larger groups (Archetti and Scheuring 2010). Our data do not show there to be an increase in the number of co-operators in larger ferns: *Monomorium* #009, the most vigorous patroller, is found more commonly in larger, rather than smaller ferns (Supplementary material Appendix 1 Fig. A2). Nor is it is clear from our data whether the reduction in herbivory with increasing resident ant biomass is reaching an asymptote, although the measured range of ant biomasses might not be wide enough to see this effect (Fig. 2), but we do appear to have evidence of mixed strategy in terms of the degree of protection.

It seems likely that the levels of protection received by *Asplenium* are currently by-products of ant foraging rather than investment in fern protection per se. Firstly, protection was obtained from a suite of ant species, some of which were relatively rare and none of which were present in more than 18% of the ferns. Furthermore, removing the biomass of the only individual species to negatively affect herbivory rates in its own right (which was also the most widespread species)

did not disrupt the overall relationship between ant biomass and herbivory levels. Secondly, while conducting fieldwork, touching or breaking fern fronds did not elicit aggressive reactions from resident ants, which contrasts with many other ant–plant mutualisms in which resident ants aggressively defend their host plants (Edwards et al. 2007). Because protection is a by-product benefit of normal levels of foraging activity by a range of ant species, there appears to be little chance that the invasion of non-protecting (or non-foraging) 'free-riders' would disrupt overall protection.

We then find that the public good of housing is positively related to the number of ant colonies (Fig. 3, Supplementary material Appendix 1 Table A3), but not the size or reproductive output of individual ant colonies (Supplementary material Appendix 1 Fig. A3), indicating that benefits of protection are of 'common interest' (Leimar and Hammerstein 2010) because it is new colonies that gain from increased fern size. There might be undetected benefits at the individual colony level if fern protection increases fern survival, or if fern protection increases fern fitness and local recruitment allowing the progeny of protecting colonies to benefit with increased potential for fern colonization ('spatial structuring'; Szilagyi et al. 2009). However, it appears that in this multi-visitor system, feedback benefits would not be accrued by any ant colony that was to invest in increased protection for the host-fern.

*Asplenium* ferns apparently lack a partner choice or partner fidelity feedback mechanism for decreasing non-protector fitness or for rewarding protectors, because ants do not inhabit fern-grown structures that could form the basis of such a mechanism. In contrast, these mechanisms in other ant–plant systems are based upon such plant-grown structures (Archetti et al. 2011, Brouat et al. 2001, Edwards et al. 2006). Our results thus indicate that the lack of any such mechanism leads to public goods being shared by multiple species, thus shifting the cost-benefit ratio away from reciprocal investment by partners (Fig. 3, Supplementary material Appendix 1 Table A3). Indeed, in this study, we found no circumstantial evidence to suggest that there had been any investment in the protection of host-ferns by ants beyond that which results from background levels of foraging, and ferns did not provide food sources for resident ants, as occurs in many other one host-one ant colony systems (Gibernau et al. 2007). This study therefore provides evidence that in multiple species interactions the lack of a mechanism for partitioning the public good inhibits the evolution of mutualism from the starting point of by-product benefits.

Interestingly, the lack of a mechanism to direct benefits to cooperating species in this study correlates with the persistence of high fern–ant diversity. Because the *Asplenium* do not appear to direct benefits to any one ant species, all ant species can benefit from the persistence of the public good of housing. In contrast, ant-inhabited epiphytes that invest in mutualistic relationships with host-ants typically support less species-rich ant communities (Supplementary material Appendix 1 Fig. A1). For example, the *Myrmecophila christinae* orchid provides both domatia and extra floral nectaries (Dejean et al. 2003), but these plants support relatively few ant species.

In conclusion, our work demonstrates that the evolution of mutualism in these highly diverse fern-dwelling ant communities is fixed at the stage of a non-specific two-way by-product mutualism. Plants and ants both benefit from the association, but reciprocal investment in partners appears to be precluded by the lack of a mechanism to direct benefits for better cooperators. We finish by noting that despite the apparent lack of benefit at the individual colony level, there are clear benefits to both ants and plants at the level of the public good, with possible implications for our understanding of 'common interest' (Leimar and Hammerstein 2010). Our empirical understanding of common interest in the evolution of mutualism is limited and we suggest that the study of multiple species systems, such as our *Asplenium*–ant interaction, could provide invaluable insights into public goods and how they impact upon the selection of assemblage-level traits of common interest.

# References

Archetti, M. and Scheuring, I. 2010. Coexistence of cooperation and defection in public goods games. – Evolution 65: 1140–1148.

Archetti, M. et al. 2011. Let the right one in: a microeconomic approach to partner choice in mutualisms. – Am. Nat. 177: 75–85.

Brouat, C. et al. 2001. Plant lock and ant key: pairwise coevolution of an exclusion filter in an ant–plant mutualism. – Proc. R. Soc. B 268: 2131–2141.

Chao, A. et al. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. – Ecol. Lett. 8: 148–159.

Colwell, R. K. 2009. EstimateS: Statistical estimation of species richness and shared species from samples. Ver. 7.52. User's Guide and application published online.

Cribari-Neto, F. and Zeileis, A. 2010. Beta regression in R. – J. Stat. Software 34: 2.

De Mazancourt, C. et al. 2005. Understanding mutualism when there is adaptation to the partner. – J. Ecol. 93: 305–314.

Dejean, A. et al. 2003. Nest site selection by ants in a flooded Mexican mangrove, with special reference to the epiphytic orchid *Myrmecophila christinae*. – J. Trop. Ecol. 19: 325–331.

Douglas, A. E. 2010. The symbiotic habit. – Princeton Univ. Press.

Edwards, D. P. 2009. The roles of tolerance in the evolution, maintenance and breakdown of mutualism. – Naturwissenschaften 96: 1137–1145.

Edwards, D. P. and Yu, D. W. 2007. The roles of sensory traps in the origin, maintenance, and breakdown of mutualism. – Behav. Ecol. Sociobiol. 61: 1321–1327.

Edwards, D. P. et al. 2006. Selection for protection in an ant–plant mutualism: host sanctions, host modularity, and the principal-agent game. – Proc. R. Soc. B 273: 595–602.

Edwards, D. P. et al. 2007. Protection in an ant–plant mutualism: an adaptation or a sensory trap? – Anim. Behav. 74: 377–385.

Edwards, D. P. et al. 2010. Lack of a host sanction correlates with prolific cheating in an ant–palm symbiosis. – Oikos 119: 45–52.

Ellwood, M. D. F. et al. 2002. Canopy ferns in lowland dipterocarp forest support a prolific abundance of ants, termites and other invertebrates. – Biotropica 34: 575–583.

Fayle, T. M. et al. 2009. The effect of rain forest canopy architecture on the distribution of epiphytic ferns (*Asplenium* spp.) in Sabah, Malaysia. – Biotropica 41: 676–681.

Fayle, T. M. et al. 2010. Oil palm expansion into rain forest greatly reduces ant biodiversity in canopy, epiphytes and leaf-litter. – Basic Appl. Ecol. 11: 337–345.

Fonseca, C. R. 1993. Nesting space limits colony size of the plant-ant *Pseudomyrmex concolor*. – Oikos 67: 473–482.

Foster, K. R. and Wenseleers, T. 2006. A general model for the evolution of mutualisms. – J. Evol. Biol. 19: 1283–1293.

Gibernau, M. et al. 2007. An asymmetrical relationship between an arboreal ponerine ant and a trash-basket epiphyte (Araceae). – Biol. J. Linn. Soc. 91: 341–346.

Gibson, C. M. and Hunter, M. S. 2010. Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. – Ecol. Lett. 13: 223–234.

Heil, M. et al. 2009. Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. – Proc. Natl Acad. Sci. USA 106: 18091–18096.

Huxley, C. 1980. Symbioses between ants and epiphytes. – Biol. Rev. 55: 321–340.

Kiers, E. T. et al. 2003. Host sanctions and the legume–rhizobium mutualism. – Nature 425: 78–81.

Leimar, O. and Connor, R. C. 2003. By-product benefits, reciprocity and pseudoreciprocity in mutualism. – In: Hammerstein, P. (ed.), Genetic and cultural evolution of cooperation. MIT Press, pp. 203–222.

Leimar, O. and Hammerstein, P. 2010. Cooperation for direct fitness benefits. – Phil. Trans. R. Soc. B 365: 2619–2626.

Maindonald, J. and Braun, J. 2007. Data analysis and graphics using R. – Cambridge Univ. Press.

Philpott, S. M. and Foster, P. F. 2005. Nest-site limitation in coffee agroecosystems: artificial nests maintain diversity of arboreal ants. – Ecol. Appl. 15: 1478–1485.

Sachs, J. L. et al. 2004. The evolution of cooperation. – Q. Rev. Biol. 79: 135–160.

Schoener, T. W. 1980. Length–weight regressions in tropical and temperate forest-understory insects. – Entomol. Soc. Am. 73: 106–109.

Szilagyi, A. et al. 2009. The evolution of intermediate castration virulence and ant coexistence in a spatially structured environment. – Ecol. Lett. 12: 1306–1316.

Treseder, K. K. et al. 1995. Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. – Nature 375: 137–139.

Turner, E. C. and Foster, W. A. 2006. Assessing the influence of bird's nest ferns (*Asplenium* spp.) on the local microclimate across a range of habitat disturbances in Sabah, Malaysia. – Selbyana 27: 195–200.

Turner, E. C. and Foster, W. A. 2009. The impact of forest conversion to oil palm on arthropod abundance and biomass in Sabah, Malaysia. – J. Trop. Ecol. 25: 23–30.

Turner, E. C. et al. 2007. The impact of bird's nest ferns on stemflow nutrient concentration in a primary rain forest, Sabah, Malaysia. – J. Trop. Ecol. 23: 721–724.

West, S. A. et al. 2002. Sanctions and mutualism stability: why do rhizobia fix nitrogen? – Proc. R. Soc. B 269: 685–694.

Weyl, E. G. et al. 2010. Economic contract theory tests models of mutualism. – Proc. Natl Acad. Sci. 107: 15712–15716.

Yu, D. W. and Davidson, D. W. 1997. Experimental studies of species-specificity in *Cecropia*–ant relationships. – Ecol. Monogr. 67: 273–294.

Yu, D. W. et al. 2004. Experimental demonstration of species coexistence enabled by dispersal limitation. – J. Anim. Ecol. 73: 1102–1114.