

Why are there more arboreal ant species in primary than in secondary tropical forests?

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Summary

1. Species diversity of arboreal arthropods tends to increase during rainforest succession so that primary forest communities comprise more species than those from secondary vegetation, but it is not well understood why. Primary forests differ from secondary forests in a wide array of factors whose relative impacts on arthropod diversity have not yet been quantified.
2. We assessed the effects of succession-related determinants on a keystone ecological group, arboreal ants, by conducting a complete census of 1332 ant nests from all trees with diameter at breast height ≥ 5 cm occurring within two (unreplicated) 0.32-ha plots, one in primary and one in secondary lowland forest in New Guinea. Specifically, we used a novel rarefaction-based approach to match number, size distribution and taxonomic structure of trees in primary forest communities to those in secondary forest and compared the resulting numbers of ant species.
3. In total, we recorded 80 nesting ant species from 389 trees in primary forest but only 42 species from 295 trees in secondary forest. The two habitats did not differ in the mean number of ant species per tree or in the relationship between ant diversity and tree size. However, the between-tree similarity of ant communities was higher in secondary forest than in primary forest, as was the between-tree nest site similarity, suggesting that secondary trees were more uniform in providing nesting microhabitats.
4. Using our rarefaction method, the difference in ant species richness between two forest types was partitioned according to the effects of higher tree density (22.6%), larger tree size (15.5%) and higher taxonomic diversity of trees (14.3%) in primary than in secondary forest. The remaining difference (47.6%) was because of higher beta diversity of ant communities between primary forest trees. In contrast, difference in nest density was explained solely by difference in tree density.
5. Our study shows that reduction in plant taxonomic diversity in secondary forests is not the main driver of the reduction in canopy ant species richness. We suggest that the majority of arboreal species losses in secondary tropical forests are attributable to simpler vegetation structure, combined with lower turnover of nesting microhabitats between trees.

Key-words: diversity partitioning, Formicidae, habitat disturbance, insects, species density, tree canopies, tropical ecosystems

Introduction

Most extant arthropod species live in tropical rainforests, with the forest canopy being particularly diverse (Basset *et al.* 2003; Ozanne *et al.* 2003). Despite this outstanding share of global biodiversity, we know relatively little about the processes that structure canopy species diversity and distribution

(Hammond 1992; Basset *et al.* 2003; Ellwood & Foster 2004; Tanaka, Yamane & Itioka 2010). Such knowledge is crucial as tropical forests are increasingly being converted into structurally simpler and less diverse habitats (Primack & Corlett 2005; Cayuela *et al.* 2006), with poorly understood consequences for their arthropod biodiversity (Bihn *et al.* 2008).

Ants are one of the most ecologically important animal groups in many terrestrial ecosystems because of their high

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abundance and complex role as ecosystem engineers, predators, herbivores, decomposers and seed-dispersal agents (Hölldobler & Wilson 1990; Folgarait 1998). Consequently, they play crucial roles in the structuring of plant and animal interactions over both evolutionary and ecological time-scales (Moreau *et al.* 2006). These attributes, coupled with a solid taxonomic foundation and sensitivity to environmental changes, make ants ideal group for ecological studies (Folgarait 1998; Agosti *et al.* 2000).

In tropical rainforest canopies, ants represent 20–60% of the entire arthropod biomass and up to 90% of individuals (Floren & Linsenmair 1997; Davidson *et al.* 2003; Dejean *et al.* 2007). Despite their considerable importance in this habitat, there have been relatively few studies on the diversity and distribution of arboreal ant communities in natural systems (e.g. Floren & Linsenmair 2000; Schonberg *et al.* 2004; Fayle *et al.* 2010; Tanaka, Yamane & Itioka 2010; Yusa *et al.* 2012), with most research being focused on less diverse habitats, such as plantations and managed forests (e.g. Majer 1993; Philpott & Foster 2005; Pfeiffer, Tuck & Lay 2008).

Arboreal ant assemblages have been traditionally considered to be highly structured by competition hierarchies that sometimes result in spatial ‘ant mosaics’ (Majer 1993; review in Dejean *et al.* 2007). However, the role of neutral interactions between species and stochastic events such as colonization and extinction has recently also been recognized as important (e.g. Floren & Linsenmair 2000; Ribas & Schoereder 2002; Stuntz *et al.* 2003; Sanders *et al.* 2007). Furthermore, various abiotic and biotic factors influence the arboreal ant communities, for example, the distribution of nesting sites and food resources such as extrafloral nectaries and insect symbionts (e.g. Blüthgen, Stork & Fiedler 2004; Tanaka, Yamane & Itioka 2010; Powell *et al.* 2011). Patterns observed among tropical forests are thus often incongruent, possibly due to the limited scale of sampling and methodological differences between studies (Basset *et al.* 2003; Floren 2005).

Ecological research of plants in tropical forests relies heavily on sampling of entire vegetation plots (Condit 1995), whereas comparable data sets for insects are lacking. To the best of our knowledge, no study has exhaustively surveyed the ant community from trees in a continuous tropical forest area. Consequently, how arboreal ant diversity responds to changes in plant species diversity, tree size and nesting resources remains poorly known at level of entire forest communities, although such factors have been suggested to play crucial roles (Ribas *et al.* 2003; Campos *et al.* 2006; Tanaka, Yamane & Itioka 2010; Powell *et al.* 2011).

The diversity of ants usually declines and species composition changes predictably along disturbance gradients (Watt *et al.* 1997; Schulz & Wagner 2002; Dunn 2004; Floren & Linsenmair 2005; Fayle *et al.* 2010). However, the causal mechanisms of this decline are not fully understood. Forest disturbance causes simultaneous loss of structural heterogeneity, plant diversity and resources (Primack & Corlett 2005). Although these succession-related factors have been thought to be responsible for

lower ant diversity in disturbed forests (Schulz & Wagner 2002; Blüthgen, Stork & Fiedler 2004; Widodo *et al.* 2004; Floren & Linsenmair 2005), their relative impacts on ant species richness have not been quantified. We argue that ‘whole-forest’ inventories, that is, the complete surveys of nests in continuous forest plots, are ideal tool for investigating patterns and determinants structuring the diversity and distribution of arboreal ant communities at local scales. For instance, a plot-based approach allows direct area-based quantification and comparison of ant communities, host trees and their interactions.

In this study, a novel ‘whole-forest’ survey of felled trees and ant nests was completed in a highly diverse rainforest ecosystem in New Guinea, contrasting old-growth (primary) and early successional (secondary) forest (Whitfield *et al.* 2012b). We assess how ant diversity responds to the succession-related vegetation traits, including tree density, size and taxonomic diversity and how those factors contribute to the overall differences in species richness between the two forest types. In particular, the extensive data sets on ant communities from individual trees allowed us to construct simulated forests characterized by various tree densities, size structures and taxonomic compositions and to therefore partition the impacts of these traits on ant diversity. Our study focuses on a deceptively simple question: ‘Why are there more ant species in primary than in secondary forests in the tropics?’

Materials and methods

STUDY SITE

The study field site was located in a lowland evergreen rainforest on latosols near Wanang village in the Ramu river basin, Madang province, Papua New Guinea (100–200 m. a.s.l., 05°14’S 145°11’E), partly used for slash-and-burn agriculture (Paijmans 1976). The climate is humid with a mean annual rainfall of 3600 mm, mean air temperature of 26.5 °C and a weak dry season from July to September (McAlpine, Keig & Falls 1983).

We sampled complete arboreal ant communities from two 0.32-ha plots (40 × 80 m), each of which formed part of a larger 1-ha study plot (Whitfield *et al.* 2012b). One plot was located in an undisturbed primary forest with a canopy height up to c. 50 m and the other in a secondary forest with canopy reaching height of 25 m, which represented c. 10 years of successional vegetation growth on an abandoned food garden. The primary forest 1-ha plot included 1336 stems with diameter at breast height (DBH) ≥ 5 cm and a basal area of 29.9 m², representing 213 tree species from 130 genera and 54 families. The secondary forest 1-ha plot included 1206 stems with DBH ≥ 5 cm and a basal area of 13.6 m², representing 90 species from 67 genera and 29 families (Whitfield *et al.* 2012b).

Both plots were chosen in cooperation with the indigenous landowners who practise subsistence swidden agriculture in their forests. This partnership allowed us to sample forest plots by felling all trees without contributing to further deforestation, as the plots were already scheduled to be felled to create food gardens. On the contrary, our research provided income and job opportunities for the village community and encouraged villagers to conserve their primary

rainforest, which is under threat from commercial logging, for further research (Novotny 2010).

SAMPLING DESIGN AND MATERIAL COLLECTION

Between February and November 2007, all trees with DBH ≥ 5 cm were felled, measured, identified to species and searched for ants in the primary (trees $N = 389$) and secondary (trees $N = 295$) plots. For each tree, we measured DBH, trunk height (to first branch), crown width, crown height and total fresh leaf weight.

Every felled tree was searched intensively for ant nests and foraging individuals by three collectors from its base to the top branches. Destructive sampling enabled the cutting of branches, attached lianas and leaves and the dissecting of parts of the trunk, bark and epiphytic aerial soil to record the complete ant fauna nesting on each tree. Each nest was classified according to its location and structure into the following categories (nest sites hereafter): in aerial soil, on bark, under bark, in trunk cavity, in dead twig (≤ 5 cm in diameter), in dead branch (≥ 5 cm in diameter), in live twig, in live branch, on leaves, on/in liana and in myrmecophytic plant.

Only nesting ants (with a queen and/or immature life stages) were included in the analysis in this study. Foraging ants were not considered as these came also from nests on the ground or in surrounding vegetation (P. Klimes, unpublished data), whilst our focus in this study is on the ant diversity closely associated with individual trees, tree species and nest sites.

SPECIES IDENTIFICATION

Several individuals from each nest, including all castes, were preserved in ethanol and later sorted to genus using Bolton (1994). Morphospecies were further determined using collections at the Institute of Entomology (the Czech Academy of Science) and the Museum of Comparative Zoology (MCZ), online databases (<http://www.antweb.org>; <http://www.newguineants.org>) and with the assistance of specialist taxonomists (see Acknowledgements). DNA barcodes (sequences of COI gene) were obtained for approximately half of the species and compared with *c.* 2000 sequences already available for New Guinea species (<http://www.formicidaebol.org>). The combination of morphological and molecular data was used to define species boundaries. Tree species were identified following Whitfield *et al.* (2012a).

DATA ANALYSIS

Our analysis focused on ant assemblages on individual trees, which constitute replicated data points within each plot. The logistical demands of the whole-forest survey, comprising all trees within 0.32-ha plots, did not allow for plot replication, that is, our data used for the comparison between primary and secondary forest are pseudoreplicated (Hurlbert 1984). This is a common problem for whole-ecosystem studies and studies on super-abundant and diverse taxa. It has been argued that when large scale has priority over replication (as in this study), cautious use of inferential statistic may be acceptable (Oksanen 2001; Chaves 2010).

Predictors of ant diversity

Tree-based species accumulation curves were used to explore the relationships between ant species richness and the number of trees in each forest type (Mao Tau function). The total expected number of ant species for each community was estimated using the Chao2 index, in

ESTIMATES v. 8.2 with 100 randomisations of sample order (Colwell 2009). As we conducted complete censuses of the ant nests within the plots, this index estimates here the richness of the local species pools, rather than the number of unsampled species within each plot.

The size-related tree traits (DBH, tree height, crown and trunk height and total fresh leaf weight) were first tested as predictors of ant diversity using multiple stepwise regression. Both dependent and explanatory variables were log-transformed prior to analysis to meet the assumption of normality and homoscedasticity of residuals and to improve the linearity because of the multiplicative effect of explanatory variables (allometric dependency). As DBH was found to be the best predictor of the number of ant species on trees ($R^2 = 0.22$, $P < 0.001$) and accounted for 85% of the total variability explained by all size traits, we used it hereafter as a surrogate of tree size (see Table S1, Supporting information for full model details).

Relationships between tree size and ant diversity, and nest density and diversity of nest sites were compared between the two forest types using a GLM model of homogeneity of slopes (effects: forest type, DBH). Mean ant species richness, nest number and richness of nest sites per tree were compared between plots using ANOVA. When needed, the variables were log-transformed prior to analyses for data normality and homoscedasticity. Mean species richness per tree was also calculated separately for different tree sizes. Trees were classified into size classes based on their log(DBH), starting from the minimum value of 0.70 (for DBH = 5 cm) and using class width of 0.1625 (i.e. one-eighth of entire range: 0.70–0.87, 0.87–1.02, etc.), except for the last class that included all trees with log(DBH) > 1.62 . Analyses were performed in STATISTICA software ver. 9.1 (StatSoft 2010).

The similarity of ant communities between trees within each forest plot was characterized by the Sørensen similarity index ($S\emptyset$), ranging from zero (no ant species shared by the compared communities) to one (all species shared). The Sørensen index was used also to estimate the similarity in nest sites between trees, using nest site category instead of species.

The correlation of taxonomic distance between trees and the dissimilarity of their ant assemblages (estimated as $1 - S\emptyset$) was assessed using Mantel tests computed in zT software ver. 1.1 with 1000 runs per analysis (Bonnet & Van de Peer 2002). Trees without nests were excluded from the analysis because $S\emptyset$ is not defined for two empty samples. Taxonomic distance between pairs of trees within each plot was coded at four levels of resolution, each of which was analysed separately: (i) *full taxonomic distances* where 0 = conspecific, 1 = congeneric, 2 = confamilial, 3 = allofamilial trees; (ii) *familial distances*, 0 = confamilial, 1 = allofamilial trees; (iii) *generic distances*, 0 = congeneric, 1 = allogeneric trees; and (iv) *species distances*, 0 = conspecific, 1 = allospecific trees. Finally, Mantel test was used in the same way to assess the correlation between ant species dissimilarity and dissimilarity of nest sites between trees in each plot.

Partitioning of the differences in ant species richness

Our data on ants and trees from plots in primary and secondary forest offered a unique opportunity to examine the effect of individual forest characteristics on ant species diversity. We selected subsets of trees from the primary forest in such a way that they matched those from secondary forest in terms of tree density, size distribution and taxonomic diversity and examined the effects of these matched parameters on ant diversity. Additive partitioning of diversity was used to explain the difference in ant species richness between the primary and secondary forests, where total ant species richness per plot (S_{Total}) is defined as the sum of species richness per tree (S_{τ}) and beta diversity of species between trees (S_{β}) (Anderson *et al.* 2011).

First, we divided each study plot into three continuous 0.1-ha subplots (40 × 25 m each) and calculated the mean number of trees per 0.1 ha, as 96 (±22.2) trees in the secondary and 122 (±9.3) trees in the primary forest (Table 1). Further, we randomly selected 500 model sets of 96 trees from the entire 0.32 ha secondary forest and 500 sets of 122 trees from the 0.32 ha primary forest plots, matching thus the tree number in each set to the mean number of trees per 0.1 ha in each respective forest type. The 500 model sets of trees from secondary forest were then used as templates in the following rarefaction analyses.

The effect of tree density on ant diversity was examined by randomly reducing the number of selected trees in the primary forest data set (122 trees) to that found in the secondary forest template, that is, 96 trees. In the next step, we controlled also for tree size by selecting 96 primary forest trees such that their distribution among the DBH size classes matched the secondary forest template. Finally, we controlled for tree taxonomic diversity by selecting 96 trees whilst matching both tree size and plant diversity of secondary forest template. Specifically, we matched the relative abundance distributions of tree species within size classes, the numbers of species within each size class and the total number of tree species, genera and families across all size classes. Each of these three steps was repeated 500 times for different secondary forest template data sets. Although the identity of species, genera and families differed between primary and secondary data sets, the distribution of individual trees among taxonomic categories in the primary forest set matched very closely that in the model secondary forest set of trees (Table 1; Table S2, Supporting information). To match spatial scales for the primary and secondary data sets, and to make full use of the data available, we used randomly drawn trees (within the above constraints) from the entire 0.32-ha plots. For further details of the algorithms used and annotated R code, see Appendices S1–S2 (Supporting information).

The simulated primary forest data sets were then used to partition the difference in species diversity of ants between the model sets of

trees from the primary and secondary forests into the effect of (i) tree density, (ii) tree size and (iii) tree taxonomic diversity (Table 2). The remaining difference in species richness, unexplained by these three factors, was partitioned into differences in alpha diversity of individual trees and beta diversity (i.e. species turnover) between trees.

Results

PREDICTORS OF ANT SPECIES DIVERSITY

We sampled a total of 1332 ant nests of 99 species from 684 trees in the two 0.32 ha rainforest plots combined. The primary forest plot was almost twice as rich in ant species as the secondary forest plot with 80 species nesting on 389 trees, compared with only 42 species on 295 trees in the secondary forest plot (Table 1). No nests were found on 30% and 23% of trees in the primary and secondary plot, respectively (usually trees with DBH ≤ 15 cm).

Accumulation curves of ant species observed in both primary and secondary forest plots did not reach asymptotes. This contrasted to the Chao2 estimates of total local species richness of 89.0 (SD = 5.56) species in primary forest and 52.6 (SD = 7.7) species in secondary forest (Fig. 1). Species nesting on only a single tree (uniques) or on two individual trees (duplicates) comprised together almost half of the species in each plot as there were 19 uniques and 18 duplicates in the primary forest plot and 13 uniques and 8 duplicates in the secondary forest plot. The most common species in primary forest, *Crematogaster polita* Smith F., nested on 96 trees (25%) and the most common secondary forest species, *Camponotus* aff. *macrocephalus* (Erichson), on 112 trees (38%).

Table 1. Characteristics of primary and secondary forest vegetation and their associated ant communities in (i) the whole 0.32-ha plots, (ii) three continuous 0.1-ha subplots (mean ± SE), (iii) model sets of trees randomly selected from the 0.32-ha plots to match the mean number of trees in 0.1 ha (means for 500 selections) and (iv) randomly selected sets of primary forest trees matching the model sets from secondary forest in the number of trees, in the number and size [diameter at breast height (DBH)] of trees and in the number, size and taxonomic diversity of trees, respectively (means for 500 templates). Overall fit in tree taxonomy was achieved as follows (mean percentage match in number of taxa of simulated set compared with template over 500 replications): species = 100.0%; genera = 99.0%; families = 98.6% (See Methods and Appendix S1, Supporting information for details)

Forest	Tree number	Tree sp. richness	Tree gen. richness	Tree fam. richness	DBH per tree	Nest number	Ant sp. richness	Ant sp. richness per tree
Secondary forest								
Whole plot (0.32 ha)	295	47	33	19	11.3	580	42	1.48
Continuous 0.1-ha plot (mean ± SE)	96.0 ± 22.2	25.3 ± 3.0	17.7 ± 3.7	12.7 ± 2.2	11.6 ± 0.9	187 ± 20	25.3 ± 3.9 (a _S)	1.58 ± 0.26
Model set of trees (0.1 ha)	96	26.9	19.3	13.5	11.3	188.6	27.5 (b _S)	1.48 (f)
Primary forest								
Whole plot (0.32 ha)	389	115	78	41	12.7	752	80	1.52
Continuous 0.1-ha plot (mean ± SE)	122.0 ± 9.3	57.3 ± 3.4	46.0 ± 0.6	29.3 ± 0.7	12.8 ± 0.2	239 ± 53	47.3 ± 8.4 (a _P)	1.52 ± 0.21
Model set of trees (0.1 ha)	122	61.7	49.0	29.6	12.7	235.5	52.7 (b _P)	1.52
Selection of primary forest trees simulating the secondary model set of trees								
Matching tree density	96	52.9	43.4	27.3	12.7	184.9	47.0 (c)	1.52
Matching tree density and size	96	53.1	43.5	27.3	11.3	173.9	43.1 (d)	1.45
Matching tree density, size and taxonomy	96	27.1	19.6	13.6	11.2	176.1	39.5 (e)	1.48 (g)

Table 2. Additive partitioning of differences in ant species richness (S_{Total}) between 0.1 ha of primary (P) and 0.1 ha of secondary (S) forest (See Table 1 for the definition of parameters a–g)

Difference in S_{Total} between primary and secondary forest	Additive partitioning	Mean number of ant species
Continuous 0.1-ha plot (mean, $n = 3$)	a _P –a _S	22.0
Simulated 0.1-ha plot	b _P –b _S	25.2
Effect of tree number	b _P –c	5.7
Effect of tree size (diameter at breast height)	c–d	3.9
Effect of tree taxonomy	d–e	3.6
Effect of alpha diversity per tree (S_{α})	g–f	0.0
Effect of beta diversity between trees (S_{β})	(e–g) – (b _S –f)	12.0

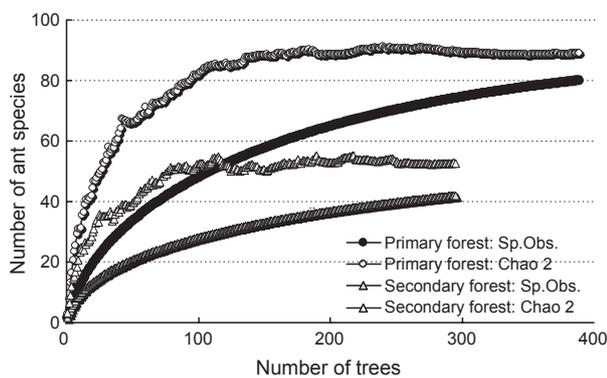


Fig. 1. Species accumulation curves for observed (Sp. Obs., Mao Tau) and estimated (Chao 2 estimator, the mean values from 100 randomisations) species richness of nesting ants in trees in 0.32 ha of primary and 0.32 ha of secondary rainforest. Individual trees are accumulated in random order.

The distribution of trees in size classes differed between the plots ($\chi^2 = 27.3$, d.f. = 6, $P < 0.001$) with secondary forest having relatively fewer of the smallest trees, more of the middle size trees and no trees with DBH over 44 cm (Fig. S1, Supporting information). Other vegetation characteristics also differed considerably between the forest plots (Table 1). However, despite these disparities, the number of ant species increased in the same way with tree size (DBH) in the two forest types (GLM, effect of forest: $F_1 = 1.27$, $P = 0.26$; Fig. 2a; Table S3, Supporting information). Similarly, there was no difference between the two forest types in the relationship between tree size and the number of nests (GLM: $F_1 = 0.65$, $P = 0.42$) and also between tree size and diversity of nest sites (GLM: $F_1 = 1.39$, $P = 0.24$) (Fig. S2 and Table S3, Supporting information).

Individual trees hosted 0–12 ant species (mean 1.52 ± 1.67) in the primary forest plot and 0–8 species (mean 1.48 ± 1.34) in the secondary forest plot. The mean number of ant species per tree did not differ between primary and secondary forest trees for all tree sizes combined ($F_{1,682} = 0.56$, $P = 0.45$). However, it varied considerably among tree size classes from one to six species per tree on average (Fig. 2b). The number of nests, 0–20 (mean 1.93 ± 2.37) per primary forest tree and 0–13 (mean 1.97 ± 2.11) per secondary forest tree, was also not

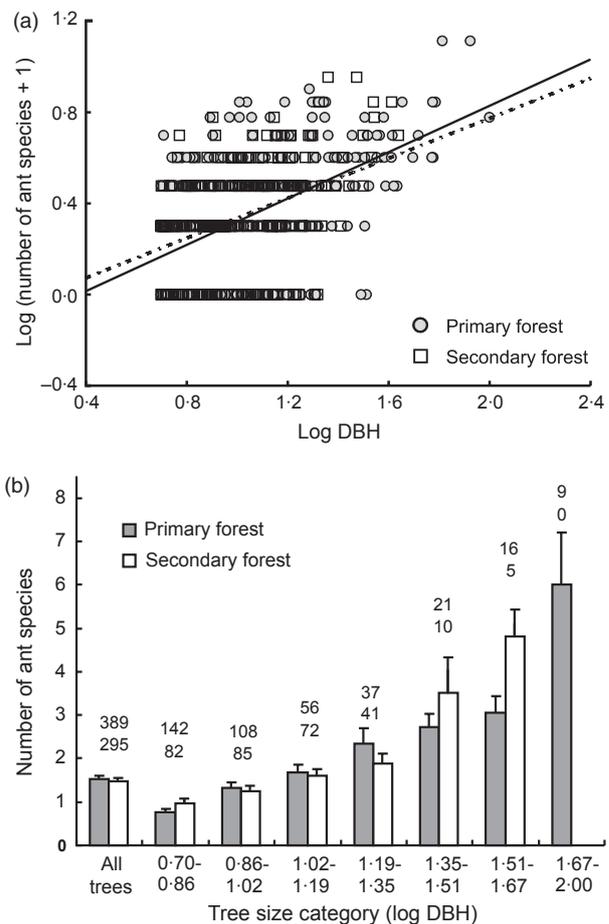


Fig. 2. Relationship between ant species richness and tree size. (a) Log-log plot of number of nesting species on the diameter at breast height (DBH) of tree trunk; primary forest, circles and full line; secondary forest, squares and dashed line. There is no significant difference in the regression slopes between the two forests (GLM: $F_1 = 1.27$, $P = 0.26$). (b) The average (\pm SE) number of nesting species per tree for all trees with DBH ≥ 5 cm and for logarithmically scaled tree size classes (see Methods for their definition). The upper and lower number above each column indicates the number of trees in primary and secondary forest, respectively. The species richness per tree does not differ between forests (ANOVA, all trees: $F_{1,682} = 0.56$, $P = 0.45$).

significantly different between the two forest types ($F_{1,682} = 1.1$, $P = 0.29$). The number of nest site categories varied from one to five per tree in the both forest types

(Fig. S2b, Supporting information) and also did not differ between primary forest (mean 1.23 ± 1.07) and secondary forest (mean 1.24 ± 0.99) ($F_{1,682} = 0.05$, $P = 0.83$).

The mean similarity of ant species composition between pairs of trees in secondary forest, $S\bar{O}_{\text{mean}} = 0.10$, was approximately double of that in primary forest ($S\bar{O}_{\text{mean}} = 0.04$). Moreover, the difference was evident in all size classes (Fig. S3, Supporting information), indicating that there is a robust pattern of higher ant species turnover between trees in primary than in the secondary forest. Similarity of ant species composition between pairs of conspecific trees was higher than between heterospecific trees in both forests (Fig. 3a). The highest mean species similarity was found between conspecific trees of the secondary forest ($S\bar{O}_{\text{mean}} = 0.14$). The similarity patterns for nest sites were analogous to those for ant species (Fig. 3b), with the highest similarity again found between conspecific trees in the secondary forest ($S\bar{O}_{\text{mean}} = 0.21$).

The taxonomic distance between trees and the dissimilarity of their ant communities were positively correlated with both forest types at all levels of taxonomic resolution: family, genus, species (Mantel test, $P = 0.001$) with exception of the familial level in primary forest (Mantel test, $P = 0.102$) (Table 3). The relationship was strongest at the tree species level in both forests, but the correlation was extremely low in all cases with weaker correlations in primary than in secondary forest (R : primary forest: range = 0.02–0.03; secondary forest: range = 0.04–0.09) (Table 3). The dissimilarity in ant species composition was positively correlated with the

dissimilarity of their nest sites in both habitat types (Mantel test, $P = 0.001$). These correlations were much stronger than those between dissimilarities of ant communities and taxonomic distances between trees (primary forest: $r = 0.22$, $P = 0.001$; secondary forest: $r = 0.24$, $P = 0.001$).

PARTITIONING OF THE DIFFERENCES IN ANT SPECIES RICHNESS

The primary forest comprised on average 122 tree individuals and 47.3 ant species within a 0.1-ha plot, whilst the secondary forest included 96 tree individuals and 25.3 ant species in the same area (Table 1). The mean ant species richness of the sets of 122 primary forest trees randomly drawn from 0.32 ha of the forest comprised 52.7 ant species whilst for the sets of 96 secondary forest trees drawn from 0.32 ha the mean was 27.5 ant species. Our simulations showed that the difference of 25.2 ant species between the primary and secondary model sets could be explained by higher tree density (5.7 species), larger tree size (3.9 species) and higher tree taxonomic diversity (3.6 species), with the remaining difference being attributable to higher beta diversity between trees (12.0 species) in primary forest, as the simulated forests did not differ in the number of ant species per tree (alpha diversity) from the secondary forest data sets (Table 2, Fig. 4). The difference in the density of ant nests was explained completely by differences in tree density between the two forest types (see values for nest numbers in Table 1).

Discussion

With 684 trees sampled in total, this is currently the most extensive study of arboreal ants from a tropical rainforest. As expected, we found a much lower diversity of ants in the secondary than in the primary forest plot. The ant species richness in secondary forest was approximately half that of in primary forest, and this difference was proportionally similar at both 0.32 and 0.1 ha scales. Previous studies also usually found large differences in species richness between primary and secondary forests (Schonberg *et al.* 2004; Floren & Linsenmair 2005; Bihn *et al.* 2008; Klimes *et al.* 2011), although less dramatic effects of human disturbance on rainforest ant diversity have been also reported (e.g. Schulz & Wagner 2002; Woodcock *et al.* 2011). However, all these studies found considerable change in ant species composition during rainforest succession. In our study, the species composition was also very different as there were only 23 shared species between the two plots, and different species dominated each forest type at the site (Klimes *et al.* 2011).

Surprisingly, and in contrast to overall diversity in plots, mean ant species richness per tree did not differ between forest plots. We expected higher species richness on primary trees than secondary trees, because the higher structural complexity of vegetation and availability of epiphytes and climbers should provide more nesting opportunities for ant species (Ribas *et al.* 2003; Campos *et al.* 2006; Turner & Foster 2009; Tanaka, Yamane & Itioka 2010). Indeed,

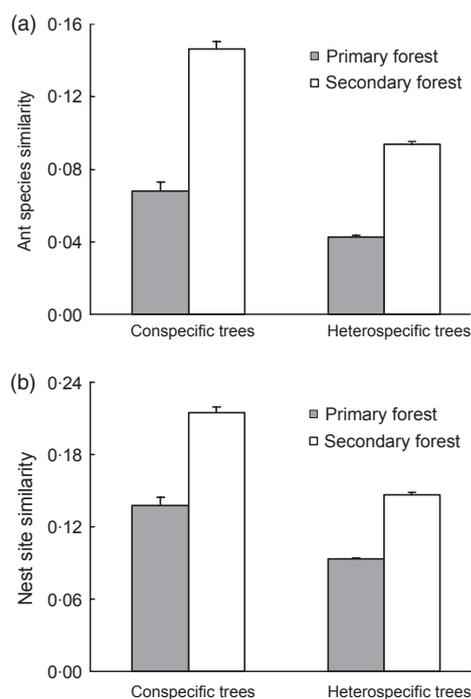


Fig. 3. Similarity in ant species composition (a) and composition of their nest sites (nesting microhabitats) (b) among conspecific and heterospecific pairs of trees in primary and secondary forest. The mean (\pm SE) value of the Sørensen similarity index was calculated for all pairs of trees with diameter at breast height ≥ 5 cm.

Table 3. Relationship of taxonomic distance between trees and the dissimilarity of their ant communities. Dissimilarity of communities (1 – Sørensen similarity index) and tree taxonomic distance at four levels of resolution (see Methods for more) were correlated for all pairs of trees with ant nests from 0.32 ha of primary and 0.32 ha of secondary forest

Primary forest	Full taxonomic level	Familial level	Generic level	Species level
Size of the matrices: 269 × 269				
<i>R</i>	0.022	0.007	0.029	0.031
<i>P</i>	0.001	0.102	0.001	0.001
Secondary forest				
Size of the matrices: 228 × 228				
<i>R</i>	0.061	0.044	0.047	0.086
<i>P</i>	0.001	0.001	0.001	0.001

Significant correlations ($P < 0.05$, Mantel test, 1000 iterations) are in bold.

Floren & Linsenmair (2005) found twice as many ant species per fogged tree in primary than in early successional rainforests. However, this pattern was not replicated in our study. As we specifically noted nesting species, we are confident that these results reflect the actual ant occupancy of studied trees. The relatively high proportion of unique species in our samples reflects genuinely low nest density of many species in trees, although some of these might be more commonly found at ground level.

The overall ant species diversity of 99 species sampled from 684 trees and the mean number of species per tree (1.5) were much lower compared with other studies of arboreal ant communities in tropical rainforests, for example, 231 spp. on 30 trees in Borneo (Floren & Linsenmair 2005) and 161 spp. on 61 trees in Africa (Schulz & Wagner 2002) and 4–40 ant species per tropical tree on average (Wilson 1987; Floren & Linsenmair 1997, 2000, 2005; Schulz & Wagner 2002; Schonberg *et al.* 2004). However, we argue that these estimates are not comparable to our study of arboreal nesting ants. First, these studies were based primarily on canopy

fogging so that their diversity estimates include also numerous ant foragers from nearby vegetation and from the ground (see Hammond 1992; Schulz & Wagner 2002; Floren 2005). Second, previous research focused on small numbers of trees of particular size, selected over a larger area, whilst we sampled entire patches of vegetation within a rather small area (two 0.32-ha plots). It is thus not surprising that species richness in our study is lower. Although comparable data are scarce, several other studies found similar or even lower number of ant nesting species per tree with DBH over 40 cm in tropical forests (Tanaka, Yamane & Itioka 2010; Janda & Konecna 2011). Another recent study reported that arboreal species were present in only 56–74% of trees in transects within a continuous rainforest (Dejean *et al.* 2010), a similar rate of occupancy to that which we found. Hence, we believe that our rainforest site is representative for arboreal ant communities of a highly species-rich tropical region such as New Guinea.

A strong positive correlation of both nest density and species richness with tree size was observed in both forest types. This was expected as tree size has been shown previously to be an important predictor of ant richness and number of colonies (e.g. Campos *et al.* 2006; Powell *et al.* 2011). Recently, Powell *et al.* (2011) also demonstrated that there is a stronger correlation between ant species richness and tree size in forests of higher tree density than in less dense forests. However, this pattern is not universal as demonstrated by our surprising result that arboreal ant diversity, nest density and microhabitat richness varied in the same way with tree size in both primary and secondary forest plots in our study, despite differences between the plots not only in tree density, but also in tree size-range distribution.

The diversity of plants is thought to be an important factor in maintaining the diversity of ant communities (Ribas *et al.* 2003; Moreau *et al.* 2006; Dejean *et al.* 2007) and insects in general (Novotny *et al.* 2006). It is also well known that in many myrmecophytic plants the composition of ant communities can differ between host tree species as a consequence of shared evolutionary plant-ant history or because of the variation in the provision of food and nesting resources (Blüthgen, Stork & Fiedler 2004; Dejean *et al.* 2007). However, myrmecophytic plants often represent only a

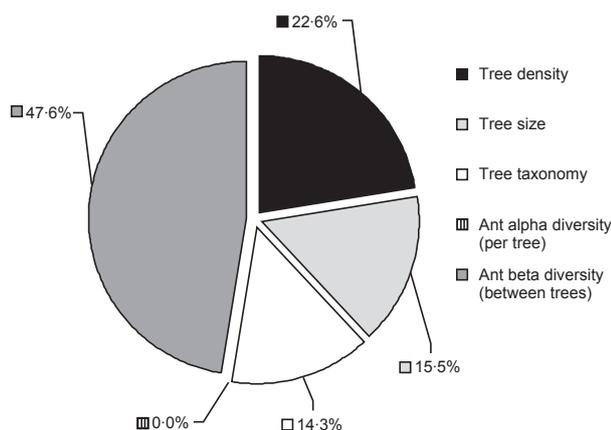


Fig. 4. The proportional difference in total ant species richness between 0.1 ha of primary forest and 0.1 ha of secondary forest due to the effects of different density of trees, tree size (diameter at breast height), taxonomic diversity of trees, ant alpha diversity per tree and ant beta diversity (species turnover between trees) between the two forest types (See Methods and Tables 1 and 2 for details on calculations).

fraction of local tree diversity and studies exploring ant fauna from locally common tree species usually find no significant effect of tree species identity on tropical ant communities (e.g. Floren & Linsenmair 1997; Schulz & Wagner 2002; Janda & Konecna 2011). Here, we demonstrate that the effect of plant taxonomic diversity on ants is significant at the 'whole-forest' level and that ant communities are more similar to each other on conspecific than on heterospecific trees. Our results also imply that arboreal ant communities are relatively more specialized to plant species (or genera) than families. Nevertheless, the overall effect of plant taxonomy was very small in both habitats suggesting that a higher taxonomic diversity of primary vegetation does not itself explain why ant diversity in primary forest is twice that of secondary forest. Our finding for arboreal ants is congruent with a recent analysis of leaf-litter fauna that also showed a low impact of plant diversity on rainforest ants (Donoso, Johnston & Kaspari 2010). Nevertheless, the future challenge is to explore also the affiliation between individual tree taxa and particular ant species relating to other environmental variables, for example, the presence of extrafloral nectaries, homopteran symbionts and spatial interactions (Davidson *et al.* 2003; Blüthgen, Stork & Fiedler 2004) and to expand our approach to include phylogeny (rather than taxonomy) of both ant and plant communities (Whitfeld *et al.* 2012b).

The differences in ant species diversity between the two forest plots were primarily due to variation in beta diversity (species turnover) of ant communities between trees as we did not find any difference in mean alpha diversity of ants per individual tree (Fig. 4). Indeed, the similarity of ant communities among primary forest trees was much lower than in secondary trees. Other studies have also noted high species turnover between rainforest trees, but they have not found any common mechanism contributing to these patterns (Floren & Linsenmair 1997, 2000; Stuntz *et al.* 2003; Yanoviak, Fisher & Alonso 2008). Here, we were able to explicitly assess the contribution of particular succession-related determinants on ant diversity by comparing the species richness between model data sets of primary and secondary forest trees. Intriguingly, our results demonstrate that even when both forest types are matched for tree abundance, tree size and taxonomic structure, there is still a much higher beta diversity of ant communities between primary forest trees than secondary forest trees, accounting for almost half (47.6%) of the difference in species richness. We suggest that this unexplained fraction probably relates to the markedly higher levels of microhabitat heterogeneity previously reported between trees in primary forests (Floren & Linsenmair 2005; Turner & Foster 2009) as we found a much higher similarity of ant nest sites among secondary trees than among primary trees (Fig. 3b). These results imply that secondary forest trees were more uniform in the nesting opportunities they provided compared with trees in primary forest, because numerous associated epiphytes and lianas increased tree-to-tree variability (microhabitat turnover) in primary forest. Indeed, large climbers (DBH \geq 5 cm) were present

only in the primary forest plot, and one of the most frequent nest sites in that habitat was aerial soil, which was not the case in the secondary forest plot (P. Klimes, unpublished data). The high importance of nesting resources for ants suggests also that the correlation in composition of ant communities with their nest site composition was much stronger than correlation with tree taxonomic composition. However, rather unexpectedly, we found the same nest density and nest site richness per tree (within-tree variability) in both primary and secondary forest. This may explain why we recorded the same alpha diversity of ants per tree in the two habitats, because canopy ant communities are thought to be limited by nest site variability and availability (Philpott & Foster 2005; Powell *et al.* 2011).

We thus did not find strong support for the conclusion of Ribas *et al.* (2003) that plant diversity and density are the most important factors for maintaining ant diversity at local scales. Analogous studies to our own from other tropical regions are needed to evaluate how the effects of these factors might vary between tropical ecosystems. Nevertheless, our results agree with those from structurally simpler environments such as savannas and coffee plantations, where nest site diversity has been shown to be important in sustaining high diversities of twig-dwelling ants (Philpott & Foster 2005; Powell *et al.* 2011).

Our results have potential implications for the conservation of rainforest ant communities. As vegetation structure was more important than tree diversity, current forest regeneration efforts (e.g. Hector *et al.* 2011) should focus specifically on recreating high canopy forest that emulates primary forest in terms of structure. For ants at least, high tree species diversity is not a priority. Indeed, selectively logged forests, in which particular tree species are removed, but the overall forest structure is left relatively unchanged, support rather similar diversity of ant communities as primary forests (Wido *et al.* 2004; Woodcock *et al.* 2011). However, other arboreal arthropods such as herbivorous larvae show much greater host tree specificity (Novotny *et al.* 2006), and so conservation priorities are likely to be different for these groups.

The data presented are exceptional in that they represent full censuses of ant nests from all trees within continuous areas of tropical forest. We argue that this type of data, although difficult to obtain, is crucial for further studies of insect communities. Likewise, our understanding of the composition and dynamics of rainforest vegetation is based to a large extent on the analysis of census data from continuous plant plots (Condit 1995; Leigh *et al.* 2004). Insect data from continuous plots allow rigorous comparisons of various aspects of insect communities between different forest types, as demonstrated here for primary and secondary forests. Nevertheless, we acknowledge that our results should be interpreted with caution, as sampling units (trees) were replicated within plots, which were not themselves replicated. The challenge for further studies is to increase the size of each plot and also to replicate plots within each forest type. In particular, the effect of tree density on arboreal ants requires further research as data from replicated plots show that stem density

varies both between individual plots of each forest type and among the secondary successional stages (Whitfeld *et al.* 2012a).

In conclusion, we have used perhaps the most complete existing data sets of arboreal ant communities to show that their species and nest density per tree scale universally with tree size in both primary and early successional tropical forests, despite large differences in their overall ant species diversity. Furthermore, we have demonstrated that approximately half of the impact of habitat conversion on ant species richness relates to decreases in tree abundance, a shift in tree size distribution and a simplification of tree taxonomic structure. The remaining effect is entirely at the level of ant beta diversity between trees and probably relates to a reduction in the turnover of nesting sites between trees. These findings suggest that plant taxonomic diversity is not the main driver of canopy ant diversity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Distribution of trees in individual size classes in primary and secondary forest.

Fig. S2. Relationships between tree size, number of ant nests and diversity of nest sites.

Fig. S3. Similarity in ant species composition between pairs of trees in primary and secondary forest for all trees together and across the seven tree size classes.

Table S1. Summary of stepwise regression of ant species richness on tree size traits.

Table S2. Distribution of trees among size classes and taxonomic categories in simulated 0.1 ha forest plots.

Table S3. Summary of GLM model of effects of the forest type, tree size and their interaction on ant species richness, nest number and nest site richness.

Appendix S1. Supplementary methods for the rarefaction algorithm for partitioning of difference in species richness.

Appendix S2. Text file providing the R code for the rarefaction algorithm.

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IN FOCUS

Unpacking the impoverished nature of secondary forests



An ant worker of the genus *Diacamma* foraging in the understorey of a lowland rain forest in Papua New Guinea. These ants belong to the species usually nesting in aerial soil in the canopy of primary forest trees (Photo and copyright: M. Janda, www.newguineants.org).

Klimes, P., Idigel, C., Rimandai, M., Fayle, T.M., Janda, M., Weiblen, G.D. & Novotny, V. (2012) Why are there more arboreal ant species in primary than secondary forests? *Journal of Animal Ecology*, **81**, 1103–1112.

In a world where even documenting species declines in tropical systems is challenging enough, Klimes *et al.* raise the bar by addressing the deceptively simple, yet inherently complex, question of why species richness is lower in secondary forests. Using the first plot-scale inventory of arboreal ant nests, combined with an innovative rarefaction technique, they quantify the relative importance of a range of successional factors and highlight the contribution of beta diversity to the higher richness in primary forest.

There is concern about the high rates of deforestation in tropical ecosystems that have resulted in an unprecedented loss of biodiversity; indeed, secondary forests are now estimated to cover more than 30% of the total tropical forest area (Chokkalingam *et al.* 2000). Overwhelming evidence indicates that these habitats are species-poor, containing lower biodiversity values than adjacent primary forest (Gibson *et al.* 2011). Disturbances, such as the conversion of forest to oil palm, result in a loss of biomass, and as a consequence, there is often considerable simplification of habitat structure (Grime 1979; Floren & Linsenmair 2005). Changes to habitat structural complexity are some of the most important and obvious direct effects of disturbance, although there are others, such as change in species composition and loss of food resources. The structure and functioning of biotic communities also can be influenced in many indirect ways linked strongly to habitat structure, including changes to microclimate, predation risk and modification of competitive interactions (Tews *et al.* 2004; Table 1).

Table 1. Direct and indirect effects of disturbance on biotic communities

Direct	Indirect
Change in habitat structure	Change in microclimate
Change in plant species composition	Change in competitive interactions
Loss of food resources	Change in predation risk
Loss of nest sites	Change in nest site availability
	Increased likelihood of stochastic events having detrimental effect/increasing extinction risk

For ants, diversity peaks in tropical forest canopies, and they dominate these systems in terms of their biomass and abundance (Hölldobler & Wilson 1990). Past work has focused on how competitive interactions (resulting in ant mosaics), resource availability and tree diversity contribute to the structure of local arboreal ant assemblages (e.g. Blüthgen *et al.* 2000; Blüthgen, Stork & Fiedler 2004). Yet others suggest there is evidence that assem-

blages in secondary and primary forests are structured differently with stochastic factors important in primary forest and deterministic processes such as competition more important in secondary forests (Floren & Linsenmair 2005). To date, however, there have been no explicit tests, and little is known about the relative importance of plausible modifiers.

In this issue, Klimes *et al.* take a closer look at the question of why secondary forests have fewer species than primary forests. Applying a novel approach combining the first-ever complete inventory of arboreal ant nests at a plot scale and a clever rarefaction technique, they were able to partition the relative contribution of different succession-related factors in explaining this difference. In a result that might surprise some ecologists, the taxonomic composition of the trees played less of a role than tree density or size. Indeed, their study instead stresses the importance of habitat heterogeneity in promoting species diversity. Although the recognition of the role of habitat complexity is not itself new, the study advances understanding of disturbance and key factors structuring assemblages in tropical forests and importantly provides information on the relative importance of these.

As part of a large-scale, intensive study across Papua New Guinea, Klimes *et al.* took the opportunity to make complete inventories of patches of forest designated for clearance; clearance of the forest vegetation was performed systematically enabling a full inventory. Thus, within two 0.32-hectare plots (one primary forest and one secondary forest), the authors searched intensively all trees and associated vegetation (e.g. lianas and epiphytes) for ant nests, with nests classified by location and structure. Addressing some of the main predictors of ant species richness, they also collected additional data on tree density, tree size and vegetation taxonomic diversity. Although the study is unreplicated, and thus variability across the forests is not quantified, this highly labour-intensive and challenging task represents a major advance on previous studies. This is because canopy-fogging studies cannot distinguish exclusively arboreal species from epigeic forager species, tend to focus only on larger trees and have limited replication. Typically, only a handful of trees are sampled, and owing to logistical constraints, fogging is rarely replicated at different times of the day; this is problematic as many arboreal ant species have distinct foraging windows, and at any one time, only a fraction of species will be sampled.

To partition the contribution of each succession-related factor, the authors controlled for the number of trees by randomly selecting a subset of 96 trees from both forest types so that sample size was equal and the number of trees in the primary forest data sets matched that found in the secondary forest sample. Size distributions of trees and tree taxonomic structure were also matched. Three simulated data sets were thus drawn from their empirical data and used to partition the difference in species diversity of ants into the effect of tree density, tree size and tree taxonomic diversity.

Although the mean number of species per tree in each forest type was the same, there was much higher beta diversity between primary forest trees than secondary forest trees, thus promoting local richness. Klimes *et al.* (2012) found the high ant beta diversity was matched by equally higher turnover of nest sites between primary forest trees, and they attribute the higher beta diversity in these forests to more complex vegetation structure, providing more nest site opportunities. Epiphytes in particular enhance the structural diversity of primary forests and are far less abundant and diverse in secondary forests; these plants play a dual role in the provision of physical niches (nest sites) and food resources (e.g. honeydew and nectar) and can thus contribute to increasing local ant species richness (Schultz & Wagner 2002; Yanoviak *et al.* 2012). Klimes *et al.* result concurs with a study from the Cerrado in Brazil where Powell *et al.* (2011) highlighted the importance of nest cavity availability and diversity for promoting arboreal species co-existence and species richness in newly colonized cavities. For twig-dwelling epigeic ants too, richness was enhanced where a diverse array of twigs was provided (Armbrecht, Perfecto & Vandermeer 2004).

Although there is some evidence to suggest that diversity of nest sites plays a role in contributing to the higher beta diversity in primary forests, Klimes *et al.* (2012) are cautious in this assertion; this is because there are many other possible explanations they did not explicitly consider. First, food resource quality, quantity and stability have long been considered important in structuring local ant assemblages, particularly where the provision of a consistent supply of carbohydrates (from honeydew and extrafloral nectaries) affects species interactions (Davidson 1997; Blüthgen *et al.* 2000; Blüthgen & Feldhaar 2010); as such, changes to the availability of food resources as a result of disturbance are likely to have profound effects on the local ant communities. Second, recent studies have highlighted the role habitat connectivity can play in promoting species diversity; Powell *et al.* (2011) found that greater canopy connectivity facilitates access to resources and increases local arboreal ant richness. The more continuous canopy cover provided by primary forest enables ants to move across the canopy, finding new food and nest resources. Third, disturbances have a major effect on microclimate (Didham & Lawton 1999; Savilaakso *et al.* 2009): daytime temperature increases and humidity decreases, while the buffering effect provided by the canopy is reduced, and consequently, diurnal variation also increases. Possible impacts on arboreal ants include a reduced foraging window, while the effect of altered microclimatic conditions on growth and development in nests is virtually unknown. Finally, the role of stochastic processes warrants further attention (e.g. Hubbell 2001; Andersen 2008); can greater niche availability in primary forest account for the higher richness, or are there more opportunities for stochastic processes affecting colonization and extinction to operate?

The study raises a number of other interesting questions about species co-existence mechanisms and the functioning of forest ecosystems. In a system where ants can constitute 85% of biomass (Hölldobler & Wilson 1990; Davidson *et al.* 2003), the finding that such a large proportion of trees did not contain any nests (a third of primary forest trees and 23% of secondary forest trees) is intriguing. Other studies have also reported a large number of 'empty' nest niches, for example, 57% of hollow epiphytic pseudobulbs were uninhabited by ants (Yanoviak *et al.* 2012), while in a manipulative experiment, Sagata *et al.* (2010) found on average only 11.2% of twigs were colonized. Although in many systems nest site cavities are typically a limited resource and consequently competition for nest sites is intense (e.g. Carroll 1979), nest site availability and colonization is complex varying with land-use type, disturbance history, biogeographical context and with the characteristics of the nest sites themselves (e.g. size, shape) (Powell *et al.* 2011). Are arboreal nest sites limited in these Papua New Guinean forests? What are the particular characteristics of the nests that make them more or less favourable for colonization (e.g. too small, too large)? To what extent do ants nesting on one tree forage widely across other trees? Or is nest site availability potentially a red herring in this instance, with other factors such as pathogens and parasites (Anderson *et al.* 2012), or recruitment limitation (Philpott & Foster 2005), possibly being more important for explaining the patchy distribution of ant colonies?

Secondary forests can differ enormously in age, land-use history and other characteristics, so it is likely that the determinants of richness will vary too (Floren & Linsenmair 2005). Klimes *et al.* (2012) examined a relatively young (10-year-old) secondary forest. Questions therefore remain as to how the relative contribution of different factors changes with successional age, and whether changes are gradual or thresholds exist (e.g. development of epiphytes provides more nest sites)?

To provide better predictions and management of biodiversity for the future, ecologists and conservation biologists need to understand both pattern and process; elucidating those mechanisms is the trickier part of the equation. Klimes *et al.* (2012) have made an important contribution to our understanding; yet as with all good studies, more questions have been generated than answered.

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