

Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics

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Received: 1 August 2014

Accepted: 4 December 2014

New Phytologist (2015) 207: 411–424

doi: 10.1111/nph.13271

Key words: ant–plant symbioses, domatia, extrafloral nectaries (EFNs), mutualism, myrmecophytes, radiations, symbioses.

Summary

- Ant–plant symbioses involve over 110 ant species in five subfamilies that are facultative or obligate occupants of stem, leaf or root domatia formed by hundreds of ant–plant species. The phylogenetic distribution and geological ages of these associations, and the frequency of gains or losses of domatium, are largely unknown.
- We compiled an up-to-date list of ant domatium-bearing plants, estimated their probable true number from model-based statistical inference, generated dated phylogenies that include c. 50% of ant–plant lineages, and traced the occurrence of domatia and extrafloral nectaries on a 1181-species tree, using likelihood and Bayesian methods.
- We found 681 vascular plants with domatia (159 genera in 50 families) resulting from minimally 158 inferred domatium origins and 43 secondary losses over the last 19 Myr. The oldest African ant–plant symbioses are younger than those in Australasia and the Neotropics. The best statistical model suggests that the true number of myrmecophytes may approach 1140 species.
- The phylogenetic distribution of ant–plants shows that domatia evolved from a range of pre-adapted morphological structures and have been lost frequently, suggesting that domatia have no generalizable effect on diversification. The Miocene origin of ant–plant symbioses is consistent with inferred changes in diet and behaviour during ant evolution.

Introduction

The fossil record and molecular clock dating show that ants and plants have been coexisting for at least 120 Myr (Brady *et al.*, 2006; Moreau *et al.*, 2006; Bell *et al.*, 2010; Magallón *et al.*, 2013; Moreau & Bell, 2013). Traits that support a long history of ant–plant interactions include elaiosomes, fatty appendages on seeds meant for ant dispersers that may have occurred as early as 75 Myr ago (Ma) (Dunn *et al.*, 2007). Extrafloral nectaries (EFNs), involving a defence mutualism through sugar secretion recruiting ant mutualists, are known from Oligocene fossils (Pemberton, 1992) and evolved over 450 times in vascular plants (Weber & Keeler, 2013). A third type of ant–plant mutualism involves ants living in myrmecophytes, plants with modified structures to host ants (domatia). No fossil ant domatia are known, nor has there been a phylogenetic analysis focusing on these structures and the geological times when they arose or were lost. For the other two ant-related plant traits, namely elaiosomes and EFNs, recent analyses suggest that they fostered diversification, implying that mutualistic interaction with insects may have impacted macroevolutionary patterns (Lengyel *et al.*, 2009; Weber & Agrawal, 2014). In the absence of a phylogenetic

framework, it is unclear whether domatia also favoured diversification.

Domatia occur in numerous plant species with modified leaves, stems or roots that provide cavities occupied by ants (Fig. 1). Some plants with domatia in addition possess specialized food bodies or EFNs. The domatium-living ants in return provide their plant hosts with protection against herbivores, with extra nutrients, or with the physical or chemical removal of competing plant species (Janzen, 1967, 1969; Davidson & McKey, 1993; Jolivet, 1996; Renner & Ricklefs, 1998). At least 113 species of ants from five subfamilies – Myrmicinae, Formicinae, Dolichoderinae, Pseudomyrmecinae, and Ponerinae – occasionally or obligatorily nest in plants (McKey & Davidson, 1993). Examples of facultative (opportunistic) ant–plant symbioses (involving domatia) are species of *Tillandsia* that can host over 30 arboreal ant species in their interlocked leaf bases (Benzing, 1970; Dejean *et al.*, 1995). Examples of obligate ant–plant symbioses are those between Central American species of *Vachellia* (formerly placed in *Acacia*) and *Pseudomyrmex* ants of the *ferrugineus* group (Heil *et al.*, 2005, 2009; Orón-Tamayo & Heil, 2013). Despite a large amount of data on aspects of chemical ecology, food webs and feedback mechanisms between plants

and ant symbionts (reviewed by Orona-Tamayo & Heil, 2013; Mayer *et al.*, 2014), little is known about the evolution of the traits that may have facilitated domatium-based symbioses and about the frequency of their evolutionary turnover in the tropical regions of Australasia, Africa and the New World where most ant–plant symbioses occur.

Phylogenetic frameworks for both the plants and the ants have so far been developed for three ant–plant symbioses, one from Africa, one from Southeast Asia and one from the Neotropics. In the African *Leonardoxa africana*, two of four subspecies have specialized domatia that were colonized in parallel by older, pre-adapted ant species (Chenuil & McKey, 1996; Brouat *et al.*, 2004). Species of the Southeast Asian *Crematogaster* subgenus *Decacrema* independently colonized three groups of *Macaranga* species: the *Pachystemon* group *c.* 12 Ma, a smooth-stemmed group *c.* 5 Ma and the *M. pruinosa* group *c.* 4.5 Ma (Quek *et al.*,

2004). As in *Leonardoxa*, colonization of plant hosts required pre-adaptations, such as the ability to adhere to slippery stems or excavation behaviour linked to specific morphological features of their hosts (Federle *et al.*, 1997, 2000; Markstädter *et al.*, 2000; Quek *et al.*, 2004). Lastly, a subgroup of Mesoamerican *Vachellia* co-diversified with *Pseudomyrmex* ants, following a single colonization event *c.* 5 Ma and subsequent host broadening within the myrmecophytic *Vachellia* (Gómez-Acevedo *et al.*, 2010). Phylogenetic studies of *Macaranga*, *Piper* section *Macrostachys*, *Neonauclea* and *Barteria* (without phylogenies of the relevant ants) have shown independent evolution of domatia within these genera, followed by secondary losses (Blattner *et al.*, 2001; Davies *et al.*, 2001; Tepe *et al.*, 2004; Razafimandimbison *et al.*, 2005; Peccoud *et al.*, 2013). Because of the need to re-associate at each generation, ant–plant symbioses likely involve little or no co-speciation but rather co-diversification, where the interacting groups



Fig. 1 Diversity of ant domatia. (a) *Myrmephytum arfakianum* (Rubiaceae), Arfak Mountains, Papua. The domatium is a swollen hypocotyle with a system of internal galleries. (b) *Hoya imbricata* (Apocynaceae), Indonesia. These so-called 'external' domatia are formed by leaves pressed against the host tree. (c) *Maieta guianensis* (Melastomataceae), Seringalzinho, Rio Jau, Amazonas, Brazil. The domatia consist of leaf pouches at the base of the lamina. (d) *Macaranga indistincta* (Euphorbiaceae) with *Crematogaster* (Myrmicinae) ants, Sabah, Borneo. Note the Beccarian bodies and the entrance holes. The inset shows a longitudinal section of an *M. pearsonii* stem domatium, showing the cultivation of scale insects by *Crematogaster* ants. Photos: (a, b) Andreas Wistuba; (c) Nigel Smith; (d) Eduard Linsenmair; inset, Brigitte Fiala.

diversify by host broadening or switching (Ehrlich & Raven, 1964; Cruaud *et al.*, 2012; de Vienne *et al.*, 2013).

Domatia might be selectively favoured in plants living in nutrient-poor habitats, such as epiphytes (Janzen, 1974), plants that already have EFNs patrolled by nectar-foraging ants, or plants patrolled by ants tending scale insects (Ward, 1991; Davidson & McKey, 1993). Wilson & Hölldobler's (2005) dynastic-succession hypothesis moreover posits that the transition from a diet involving predation on ground-dwelling insects to one involving secretions from tended hemipterans or from nectar glands, associated with aboveground living, occurred relatively late in the history of ants, coinciding with the evolution of angiosperm-dominated tropical forests that provided complex habitats. If such transitions in diet and habitat indeed evolved recently, that is, no earlier than the Eocene, then myrmecophytes inhabited by arboreal ants might be relatively young, something that can be tested with clock-dated phylogenies for relevant plant clades.

By assembling a new list of domatium-bearing vascular plant species worldwide, a large phylogenetic framework for ant-plants, and dated phylogenies for half of all myrmecophyte lineages, we address the following questions about the evolution of ant–plant symbioses: (1) How often have domatia been gained or lost (a question answerable with minimal estimates from trait reconstructions on phylogenies)? Where in the land plants do we find the highest concentrations of myrmecophyte origins and the largest clades with myrmecophytic species and how clustered are they? (2) Where are these clades located geographically? (3) How old are they? And (4) are there significant differences in the ages of myrmecophyte lineages in the Neotropics, Australasia and Africa? Such age differences might be expected because the African forests were more affected by Miocene and Pliocene climate oscillations than were Australasian and Amazonian forests (van Zinderen Bakker & Mercer, 1986; Jacobs, 2004).

Materials and Methods

Known ant-plants, types of domatia and inference of the likely total ant-plant number

In order to assemble a species-level list of ant-plants we conducted a literature search in Google Scholar (<http://scholar.google.com>) using the terms 'myrmecophytes', 'domatia', 'ant plants' and 'ant/plant symbiosis'; we also searched monographs of relevant genera, such as *Cecropia*, *Myrmecodia*, *Neonauclea*, *Triplaris* and *Ruprechtia*. We incorporated the genus-level myrmecophyte lists of Davidson & McKey (1993), McKey & Davidson (1993) and Jolivet (1996), and an unpublished list provided by Camilla Huxley-Lambrick in November 2013. The taxonomic assignment of species to genera and families was updated following recent literature and during GenBank (<http://www.ncbi.nlm.nih.gov>) searches for DNA sequences of myrmecophytes. We define a myrmecophyte as a plant species that has a structure to host ants (a myrmecodomatium); this includes external domatia (Fig. 1b), but excludes plant structures used by ants to make a nest (e.g. the root system of *Coryanthes*, Orchidaceae).

We classified domatia into eight types: (1) stem domatia, any hollow stem or twig, independent of the order or number of shoot axes transformed into domatia; (2) leaf pouches, all pouch domatia formed on the petiole and/or lamina; (3) hollow rachis, the leaf rachis axis is swollen and hollow, as in *Tachigali*; (4) leaf base domatia, a cavity formed into the spaces of interlocked leaf bases, as in *Tillandsia*; (5) stipular domatia, which include stipular thorns, stipular pouches, either closed or open; (6) root tuber domatia, for a transformed root tuber; (7) external domatia, for domatia formed by epiphytes with a structure pressed against the host tree which can be a leaf (Fig. 1b) or a modified stem; (8) hypocotyle with galleries, for the unique domatia of the Hydnophytinae (*Myrmecodia*, *Hydnophytum* and related genera).

Our list of ant-plant species is almost certainly incomplete due to overlooked literature and as yet unrecorded ant–plant symbioses. To estimate the true number of myrmecophytes, we used the model comparison framework implemented in CatchAll (Bunge, 2011). By using the same search terms ('myrmecophytes', 'domatia', 'ant plants' and 'ant/plant symbiosis') and each genus or species name from our list (Supporting Information Table S1) in Google Scholar (as of 1 September 2014), we obtained the frequency of publications per myrmecophyte species and used this as input in CatchAll. We compared five nonparametric models (Good–Turing, Chao1, ACE, ACE1 and Chao–Bunge gamma–Poisson) and five parametric models (Poisson, single exponential mixed Poisson, and mixtures of two, three and four exponentials mixed Poisson) to find the best-fitting estimate (Bunge, 2011).

Alignments and phylogenetic analyses

In order to infer the minimal numbers of gains and losses of domatia in angiosperms, we searched GenBank for the 681 myrmecophytes in our species-level list. For the 323 species present, we searched for their closest relatives, using previously published phylogenies, by including other congeneric species when genera were small, or by similarity based on the 100 highest-scoring BLAST hits of the myrmecophyte target sequence. We also included a representative sample of domatium-lacking families of angiosperms, gymnosperms and ferns, typically with one species per family except for the largest angiosperm families where one species per subfamily was included. The resulting matrix consisted of 1181 species and 3958 sequences downloaded from GenBank (<http://www.ncbi.nlm.nih.gov>), comprising the nuclear 18S rDNA and ITS regions, the plastid genes *rbcL*, *matK*, *ndhF* and *atpB*, and the plastid spacers *trnL-trnF* and *atpB-rbcL*. The final matrix comprised 1181 species and 38 080 aligned nucleotides, with 57% missing data (cells in the matrix filled with 'nnn' or '—'), including *rbcL* (799 sequences; 32% missing data), *matK* (752; 36%), *ndhF* sequences (532; 55%), *atpB* sequences (358; 69%), 18S rDNA sequences (304; 74%), ITS sequences (600; 49%), *trnL-trnF* sequences (488; 60%) and *atpB-rbcL* (135; 88%). Accession numbers are in Table S2.

Tips naming was automated with Phyutility (Smith & Dunn, 2008), and sequences were aligned with MAFFT v7 (Katoh & Standley, 2013). The five genes (*rbcL*, *matK*, *atpB*, *ndhF*, 18S rDNA) were aligned using standard settings. For the more

rapidly evolving spacer regions (ITS, *trnL-trnF*, *atpB-rbcL*), we selected the option 'leave gappy regions unaligned', with a similarity threshold of 0.8. This approach allowed us to align complete ITS sequences across land plants. Minor alignment errors were manually corrected in Mesquite v2.75 (Maddison & Maddison, 2011) and the matrices were concatenated in Geneious v5.4 (Drummond *et al.*, 2011).

Maximum-likelihood (ML) inference relied on RAxML v7.0 (Stamatakis *et al.*, 2008) with 100 ML bootstrap replicates and the analysis partitioned by gene region, all under the GTR+ Γ substitution model, as selected under the AIC criterion by jmodeltest2 (Darriba *et al.*, 2012), with six rate categories. The tree was rooted on *Selaginella moellendorffii*. The tree with all tip names is presented in Fig. S1.

Molecular clock dating of myrmecophyte groups

In order to infer absolute divergence times for myrmecophyte lineages, we generated local phylogenies that were more densely sampled than our higher-level vascular plant tree (previous section). For this, we used published datasets representing nearly half of all myrmecophyte-containing lineages: namely *Barteria* (Peccoud *et al.*, 2013), *Clerodendrum*, *Leonardoxa* (Brouat *et al.*, 2001) in Africa; *Cecropia*, *Cordia* (Weeks *et al.*, 2010), Miconiaceae (Melastomataceae; Michelangeli *et al.*, 2004), *Piper* (Tepe *et al.*, 2004), *Platymiscium* (Saslis-Lagoudakis *et al.*, 2008), *Ruprechtia*, *Triplaris* (Sanchez & Kron, 2008) and *Vachellia* (Gómez-Acevedo *et al.*, 2010) from the Neotropics; and *Dischidia*, *Hoya* (Wanntorp *et al.*, 2006), the Hydnophytinae (*Myrmecodia*, *Hydnophytum*, *Myrmephytum*, *Squamellaria*, *Anthorrhiza*), *Macaranga* (Blattner *et al.*, 2001; Davies *et al.*, 2001) and *Neonauclea* (Razafimandimbison *et al.*, 2005) from Australasia. Accession numbers are either in Table S2 or appear next to the respective species name in Figs S2–S15. Alignment and phylogenetic analyses were performed as described above for the 1181-species tree, except that the Q-INS-i approach was selected in MAFFT to take into account RNA secondary structure when aligning the ITS region, as recommended for this marker when aligning fewer than 200 sequences (Katoh & Standley, 2013).

Dating for all data matrices relied on BEAST v1.8 (Drummond *et al.*, 2012) and the GTR+ Γ substitution model with six rate categories. The tree prior was a pure-birth (Yule) tree, with MCMC chain lengths between 20 and 60 million generations, sampling every 10 000th generation, with the chain length depending on convergence as determined by examining the log files in Tracer v1.5 (Rambaut & Drummond, 2009) after removal of a burn-in proportion of 10% of the trees. Unless otherwise stated below, we used uncorrelated log-normal (UCLN) clock models. For calibration, we used either secondary constraints from other dated phylogenies or nucleotide substitution rates. Secondary constraints were assigned normal distribution priors with a standard deviation (SD) matching the 95% confidence interval from the original study when presented or otherwise a 20% SD. Specifically, the secondary calibrations were: for *Piper*, the split between *Piper* and *Peperomia* was assigned an

age of 91.2 ± 10 Myr (Smith *et al.*, 2008). For *Macaranga*, the split between *Blumeodendron* and the *Hancea* (*Mallotus* (*Macaranga*)) clade was assigned an age of 86.4 ± 5 Myr, the *Mallotus* plus *Macaranga* clade an age of 59 ± 10 Myr, and the *Macaranga* crown an age of 33.5 ± 12 Myr (van Welzen *et al.*, 2014). For *Triplaris*/*Ruprechtia*, the split between Brunnichieae and its sister clade was assigned an age of 69.1 ± 25 Myr (Schuster *et al.*, 2013). For *Platymiscium*, we set the split between *Riedellia* and its sister clade to 47.2 ± 5 Myr (node 47 in Lavin *et al.*, 2005). For *Vachellia*, we assigned the split between the (*Vachellia constricta* (*V. schottii* (*V. neovernicosa*))) clade and its sister group, which includes a myrmecophyte clade, an age of 12.3 ± 3 Myr (Gómez-Acevedo *et al.*, 2010). In the Boraginales, the relationships between the main clades were constrained to match the topology found by Weigend *et al.* (2013) with denser sampling of taxa and genes. We assigned the split between the (*Nama* (*Eriodictyon* (*Wigandia*))) clade and the rest of the Boraginales, including Cordiaceae, an age of 60.4 ± 10 Myr (Weeks *et al.*, 2010), which resulted in an age of 52 Myr for the *Ehretia* stem group, consistent with Eocene *Ehretia* fossil fruits (Gottschling *et al.*, 2002). For the Hydnophytinae (*Squamellaria*, *Hydnophytum*, *Myrmephytum*, *Anthorrhiza*, *Myrmecodia*), we assigned 14.5 ± 6 Myr to the crown group node of the sister group of Hydnophytinae (Barrabé *et al.*, 2014). For *Neonauclea*, we assigned an age of 40 ± 10 Myr to the root, corresponding to the crown group of the Cinchonoidae (Bremer & Eriksson, 2009). For *Barteria*, we assigned the split of *Barteria* and *Passiflora* to 39 ± 10 Myr using the *Passiflora* stem group age (Hearn, 2006).

For clades that lack fossils and have not been clock-dated in other studies, we used published substitution rates for calibration and strict or relaxed clock models following analyses of the extent of rate heterogeneity in Tracer. Because rates can vary greatly and may correlate with generation time (Kay *et al.*, 2006; Smith & Donoghue, 2008), we used three rates for each phylogeny, spanning the range of plausible rates. For *Leonardoxa* and the Miconiaceae, we used rates of 1×10^{-9} , 2×10^{-9} , or 3×10^{-9} substitutions per site per year, representative of ITS in woody species (Kay *et al.*, 2006), with a strict clock model for *Leonardoxa* and UCLN relaxed clocks for *Neonauclea* and the Miconiaceae. For *Clerodendrum*, we used a strict clock model and rates of 1×10^{-9} , 2×10^{-9} , or 3×10^{-9} substitutions per site per year for both ITS and the *trnL-F* region (Chase *et al.*, 1993; Richardson *et al.*, 2001; Kay *et al.*, 2006). To calibrate the Apocynaceae matrix of Wanntorp *et al.* (2006), which consists of two plastid spacer regions and nuclear ITS, we used a strict clock and rates of 2.5×10^{-9} , 3.5×10^{-9} , or 4.5×10^{-9} substitutions per site per year, consistent with noncoding plastid and ITS substitution rates in other herbaceous perennials (Manen & Natali, 1995; Richardson *et al.*, 2001; Kay *et al.*, 2006). For *Cecropia*, we built a combined *trnL-F*, *rbcL* and *matK* matrix and used a strict clock with substitution rates of 0.8×10^{-9} , 1.2×10^{-9} or 2×10^{-9} substitutions per site per year, based on rates for these loci in other woody groups (Chase *et al.*, 1993; Richardson *et al.*, 2001; Lavin *et al.*, 2005).

We cross-validated age estimates against those from published studies with overlapping taxon sampling. The trees obtained

from each clock run were summarized with TreeAnnotator v1.8.0, with a 10% burn-in and showing only nodes ≥ 0.98 posterior probability. Time-calibrated trees are shown in Figs S2–S15.

Sister-group geographic mapping

We selected 20 sister clade pairs from our 1181-species tree or published phylogenies and then downloaded the geographic ranges of these closest relatives from the Global Biodiversity Information Facility (GBIF) (<http://www.gbif.org/species>). The closest relatives were *Acacia cochliacantha*, *Adenia cynachifolia*, *Androsiphon adenostegia*, *Conceveiba pleistemonia*, *Cordia collococa*, *Cordia ecalyculata*, *Cuviera subuliflora*, *Euphronia guianensis*, *Henriettea succosa*, *Korthalsia jala*, *Leucosyke australis*, *Ludekia borneensis*, *Mallotus brachythyrus*, *M. nudiflorus*, *M. ficifolius*, *Macbridenia peruviana*, *Microsorium linguiforme*, *Piper aequale*, *Psychotria hawaiiensis* and *Ruprechtia triflora*. The distributions were plotted on a world map using DIVA-GIS (Hijmans *et al.*, 2005). The mean annual temperatures were downloaded from WorldClim (<http://www.worldclim.org/>).

Ancestral state reconstructions

In order to reconstruct gains and losses of domatia, we scored domatium absence (0) and presence (1) for all 1181 species in our tree based on our World myrmecophyte list (Table S1). Ancestral reconstruction relied on maximum likelihood (ML) implemented in Mesquite using the highest scoring likelihood tree and the Markov two-parameter model (Lewis, 2001), which allows for different forward and backward change frequencies. Domatium presence in a common ancestor was assumed if the ML probability was $\geq 70\%$. We added a single gain for genera with domatium-bearing species (Table S1) that were not included in our 1181-species matrix.

We also inferred the evolution of extrafloral nectaries on our 1181-species tree, using the same approach. We scored EFN-bearing species as 1, and EFN-lacking species as 0, according to the World List of plants with extrafloral nectaries (Keeler, 2008). We also mapped EFNs onto the *Macaranga* and the *Vachellia* chronograms. In addition to the ML approach implemented in Mesquite, we inferred ancestral states (both for EFNs and

domatia) in *Vachellia* and *Macaranga* using the Bayesian reversible-jump MCMC approach for discrete characters implemented in BayesTraits (Pagel & Meade, 2007) on a sample of 2000 trees from BEAST (burn-in excluded), thereby taking into account topological uncertainty. The chain was run for 50×10^6 generations, and rate coefficients and ancestral states were sampled every 1000th generation. We ensured that the acceptance rate was between 20% and 40% as recommended in the manual.

Results

Frequency and geography of ant–plant symbioses, and the distribution of domatium types and growth forms

Our world list of myrmecophytes includes 681 species in 159 genera and 50 families (Table S1, which also provides information on geographic ranges). Our modelling approach to estimate the true number of myrmecophytes (including ones not yet documented or missed in our literature search) yielded 1139 species under the best-fit model (1-exponential mixed Poisson, Table 1).

Ant–plant symbioses are almost exclusively tropical. Exceptions are species of *Vachellia* ranging into South Texas and African *Vachellia drepanolobium* south of the Tropic of Capricorn. There are strong diversity asymmetries in absolute species numbers, with overall *c.* 7 times more ant–plant species than plant–ant species. This asymmetry is present in all three biogeographic regions (Fig. 2) and may be strongest in Australasia, although that might be an artefact of the lack of taxonomic knowledge of Australasian ants and cryptic species complexes (personal communications from M. Janda, Czech Academy of Sciences, October 2013, and V. Witte, University of Munich, June 2014). Closest relatives of ant–plant clades for which we could evaluate geographic ranges were all distributed in the tropics and absent from temperate regions (Fig. 2).

Domatium-bearing plants are present in one family of ferns, absent in gymnosperms, and generally widespread in angiosperms, although they are absent in basal eudicots. The higher eudicots, however, contain the majority of myrmecophytes, with Rubiaceae having the highest number (162 species, Table S1), followed by Melastomataceae (144 species, Table S1). The ancestral reconstruction implies 158 independent origins and 43 losses of domatia (Fig. 3). In some genera, such as *Cecropia*, *Dischidia*,

Table 1 Predicted total number of ant domatium-bearing species from model estimates

Model		Tau	Estimated total species	SE	Lower CB	Upper CB
Best model	1 exponential mixed Poisson	8	1139	40	1067	1224
Model 2a	Poisson	5	805	16	778	840
Model 2b	2 exponential mixed Poisson	10	1160	72.8	1037	1325
Model 2c	2 exponential mixed Poisson	40	1159	49.5	1072	1267
Non-P1	Chao1	2	842	28.4	795	908
Non-P2	ACE1	10	1070	59.6	969	1205

Tau is the upper frequency cut-off; SE, the standard error of the estimate; Lower and Upper CB, the 95% confidence bound. The best model (first line) is followed by the three next best-fit parametric models (Models 2a–c) and the two best-fitting nonparametric models (non-P1, P2).

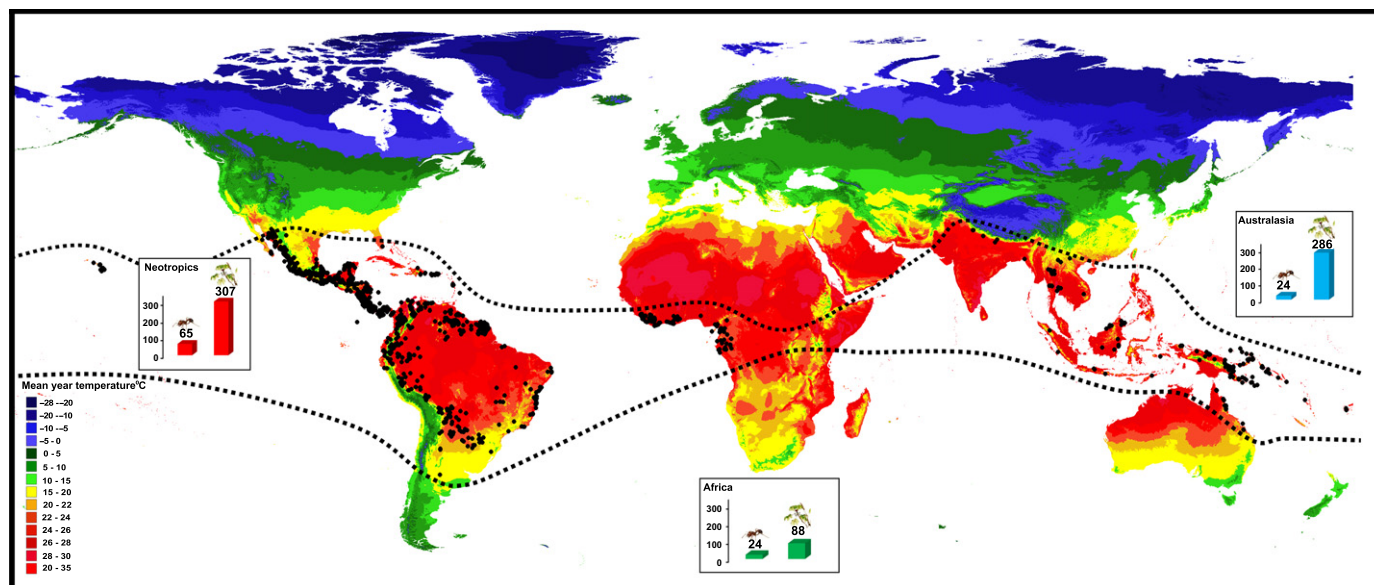


Fig. 2 Distribution of myrmecophyte sister groups on a map showing the mean annual temperature (WorldClim). The dots show the distribution of 20 species sister to over half of all 681 myrmecophytes. The dashed lines show the northern and southern borders of myrmecophyte sister group distribution. The inset shows the numbers of myrmecophytes and plant-ants in the three biogeographic regions based on our species list (Supporting Information Table S1).

Macaranga, *Neonauclea*, *Piper*, *Platymiscium* and *Tococa*, domatia evolved several times (Figs 3, S3, S6, S9–S13). In the Miconiaceae (*Tococa* and relatives; Fig. S10), leaf pouch domatia have been gained at least eight times and lost seven times, whereas in *Barteria*, *Myrmecodia* and *Triplaris* domatia appear to have evolved but once (Figs 3, S2, S7, S14). Overall, gains were over three times more frequent than losses, which may partly be due to under-sampling of myrmecophyte-rich genera that might include additional losses (e.g. *Cecropia*, *Hydnophytum*).

Stem domatia make up more than half of all domatium types (354 of 681) and were the most common type in all three biogeographic regions. Stem domatia also evolved and were lost most often (Fig. 4c). Leaf pouches are found in the Neotropics and in Africa, but are almost absent in Australasia. Hollow rachis and leaf base domatia are the particular domatium types of *Tachigali* and *Tillandsia*, respectively, and hence are restricted to the Neotropics. Australasian epiphytes, such as the ferns *Platycerium* and *Lecanopteris mirabilis* or the Apocynaceae *Hoya imbricata* (Fig. 1b), typically have external domatia, whereas Hydnophytinae (*Anthorrhiza*, *Hydnophytum*, *Myrmecodia*, *Myrmephytum* (Fig. 1a), *Squamellaria*) have swollen hypocotyls with ant galleries (Fig. 4).

Most Neotropical and African ant-plants are trees or shrubs with 79% (239 species) and 95% (83 species), respectively (Fig. 4b). By contrast, trees and shrubs make up only 40% (113 species) of the Australasian ant-plants, whereas epiphytes contribute 47% (131 species). In Africa, epiphytic ant-plants are lacking, and in the Neotropics they represent < 15%. Lianas are a rare growth form among myrmecophytes, especially in the Neotropics (0.3%), but also in Africa (3%) and Australasia (10%). Herbs are also infrequent, with the Neotropics having the highest percentage (6%), especially due to species of *Piper* (Tepe *et al.*, 2004).

Ages of domatium-bearing plant clades

In order to infer when the oldest extant domatia may have originated, we generated dated phylogenies for clades that together include 56% of the 681 known domatium-bearing species. These clock-dated lineages represent 45 independent acquisitions of domatia (Figs S2–S15); their ages are plotted in Fig. 5(a). Regardless of whether ‘slow’, ‘standard’ or ‘high’ substitution rates were used for calibration (see the Materials and Methods section), no ant-plant crown age (or stem age for single myrmecophyte species) is older than 19 Myr, and the oldest domatium-bearing species or species groups in Africa date only to the last 6 Myr. An exception is the split of *Clerodendrum rotundifolium* from its nondomatium-bearing sister group, which was dated to 10.5 Myr using the slowest of the three employed substitution rates (Figs 5a, S8). The recent origin of African myrmecophytes matches the significantly smaller species numbers in African myrmecophyte radiations (maximal radiation size evaluated by the sum of myrmecophytes per genus, Fig. 5b) as compared to Australasian and New World radiations (Fig. 5b; *t*-test, $P < 0.01$).

Discussion

Recurrent entry into a new adaptive zone by plants with ant domatia

Ant-plant symbioses are an almost exclusively tropical phenomenon (Fig. 2). That the sister groups of myrmecophyte lineages also occur in the tropics confirms that these symbioses evolved there, rather than arising by immigration of facultative ant-plant mutualisms from higher latitudes. The only extratropical domatium-occupying ants are species in the *Pseudomyrmex ferrugineus*

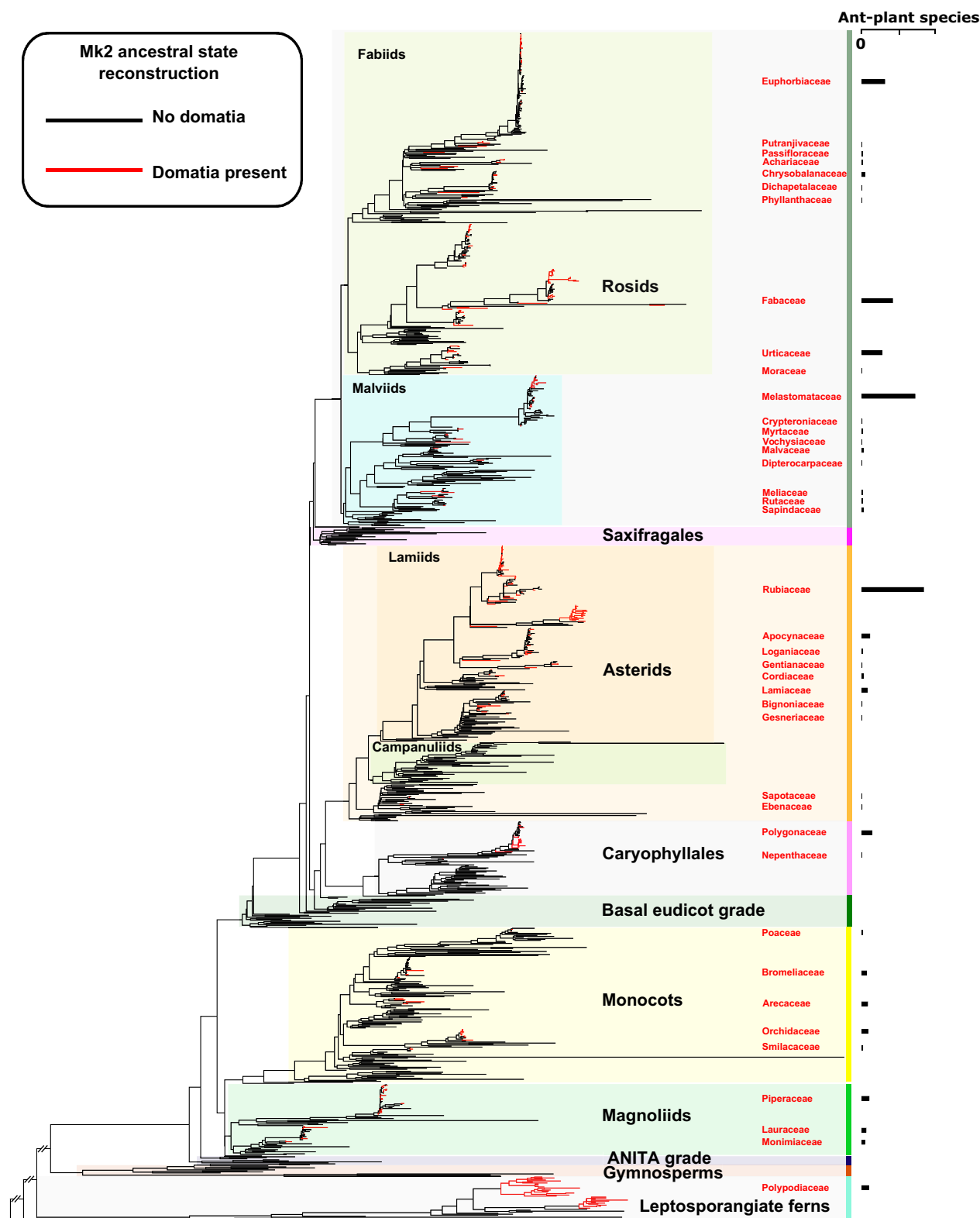


Fig. 3 Gains and losses of domatia in plants, inferred using a 2-parameter Markov model on a maximum likelihood 8-gene tree of 1181 species oversampled for myrmecophytes. Families with ant-plants in red, with the number of their ant-plant species on the right. Species names at tips are shown in Fig. S1.

group and some *Crematogaster*. The still scarce DNA sequence data for ants precluded detailed phylogenetic evaluation of the origins of plant-living ants, but the 31 ant genera known to nest

in domatia provide a minimum bound (Fig. S16). In the subfamily Pseudomyrmecinae, in which *c.* 40 of *c.* 230 species nest in domatia, Ward & Downie (2005, with a sampling of 49 of 230

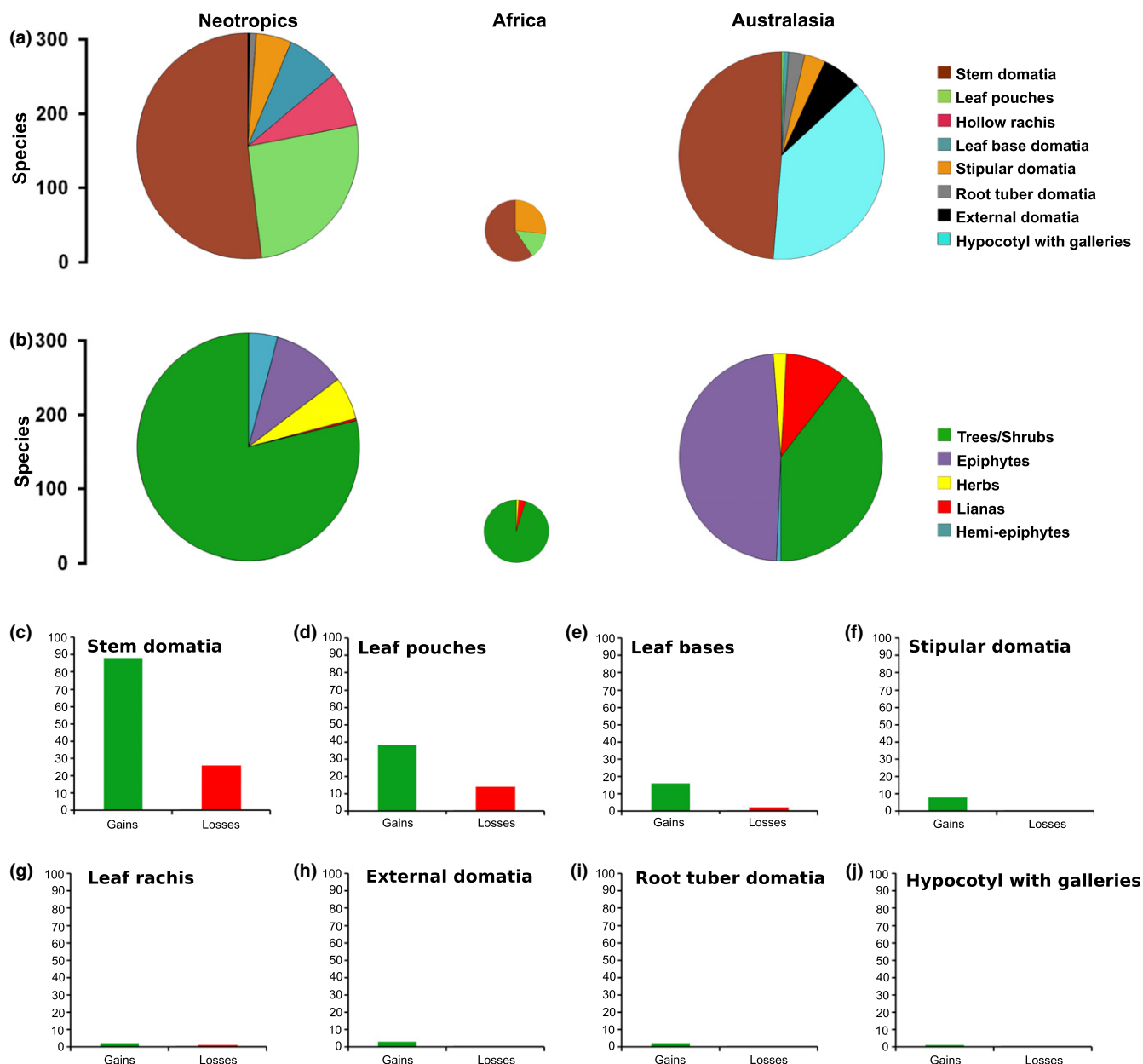


Fig. 4 Distributions and evolution of domatium types, and growth habit of myrmecophytes. (a) Distribution of domatium types in the different biogeographic regions. (b) Myrmecophyte habit. (c–j) Evolutionary gains and losses of domatium types, as inferred from the maximum likelihood (ML) reconstruction on the large tree (Fig. 3).

species) inferred 10 independent transitions to obligate domatium nesting. Together with the 31 ant genera, this yields *c.* 40 independent transitions towards obligate nesting in domatia.

The roughly three-fold higher number of domatium gains (158) than plant-ant origins (40–60) implies that recruitment of new myrmecophyte lineages through expansion of ants to non-myrmecophyte plants that subsequently evolved domatia is a driver for the evolution of additional myrmecophytes. Host switching or broadening had been inferred between species of *Macaranga* (Quek *et al.*, 2004) and *Vachellia* (Gómez-Acevedo *et al.*, 2010), but not between phylogenetically distant plant groups. Whatever the predominant modes of new host recruitment, ant-plant symbioses do not appear to have led to particular

species richness in the involved plant or ant lineages (although we conducted no formal tests). In the few ant-plant clades with high species number (*Hydnophytinae*, *Neonauclea*, *Macaranga*), it is possible to envisage higher diversification rates, but the global scatter of domatium evolution suggests that presence of this trait did not significantly enhance plant diversity. Of the 158 origins of domatia inferred in this study, most are associated with small radiations (species-poor clades) or no radiation (Fig. 5b). This implies that specialized ant-plant symbioses either represent an evolutionary 'dead-end' and go extinct, or that domatia are lost as readily as they are gained (Peccoud *et al.*, 2013). Domatium loss has been inferred in *Macaranga* (Blattner *et al.*, 2001; Davies *et al.*, 2001; Fig. S9), *Neonauclea* (Razafimandimbison *et al.*,

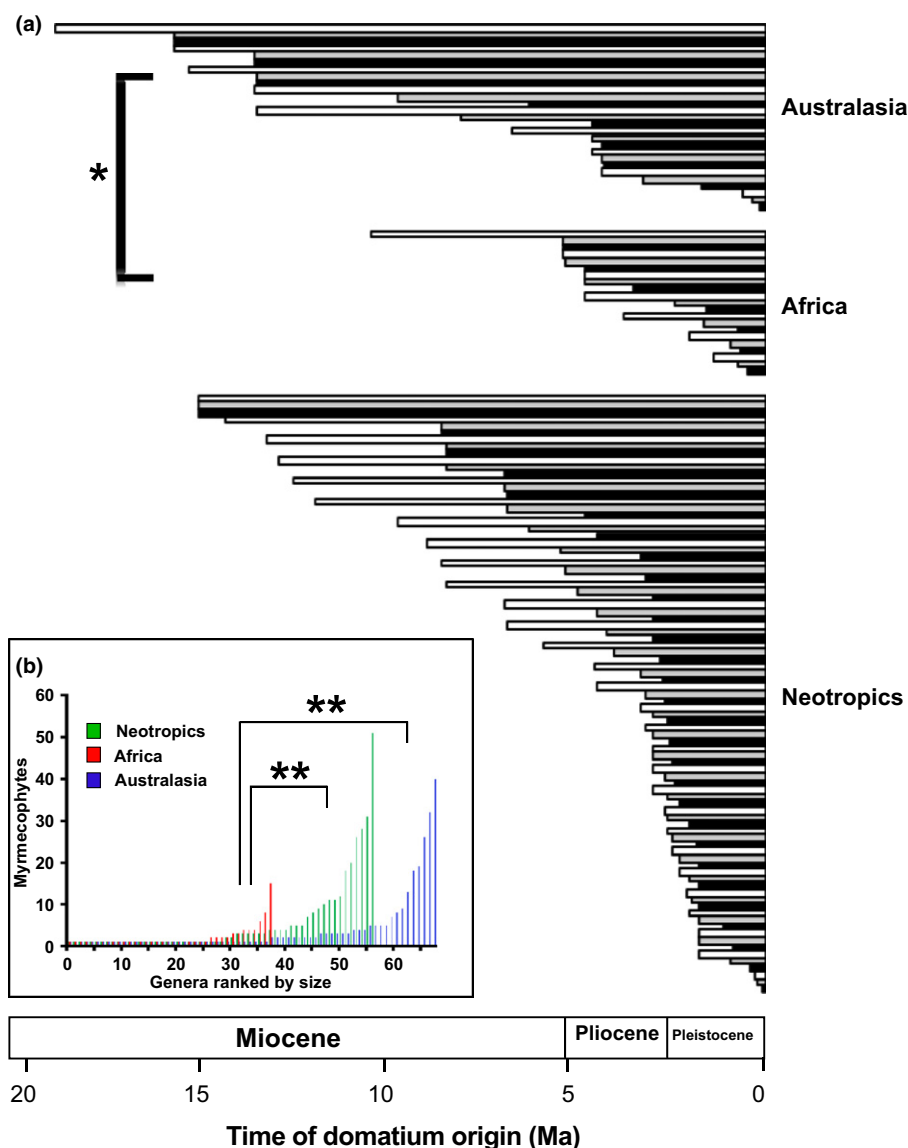


Fig. 5 Temporal build-up of myrmecophytes and maximal size of ant plant radiations in the Neotropics, Africa, and Southeast Asia/Australia. (a) Oldest inferred appearances of domatia in the three regions. Each bar represents a crown age for a node inferred to represent independent evolution of domatia. Black, grey and white shows the 'slow', 'standard' and 'high' substitution rates approaches, respectively, for lineages lacking fossil or secondary calibrations (see the Materials and Methods section). Ma, Myr ago. (b) Number of myrmecophyte species per genus in the three regions, suggesting the maximal sizes of ant-plant radiations. Asterisks refer to statistical differences in *t*-tests in (a) and (b): *, $P < 0.05$; **, $P < 0.01$.

2005; Fig. S11), *Tococa* (Michelangeli *et al.*, 2004; Fig. S10) and *Barteria* (Peccoud *et al.*, 2013), and we here inferred 43 further losses. Nevertheless, the minimally 158 gains of domatia show the recurrent entry of plant lineages into a new adaptive zone *sensu* Ehrlich & Raven (1964). The apparent evolutionary lability of domatia, with for instance minimally eight gains and seven losses in the Miconieae alone (Figs 1, S10), matches their phenotypic plasticity. For example, in *Tococa*, the same individual can possess or lack leaf pouch domatia (Renner, 1997), suggesting that domatium development, at least in the Miconieae, may depend on labile gene expression switches.

Scattered phylogenetic pattern of ant plants

An important result of this study is the phylogenetic scatter of domatium-bearing species. This differs from other findings of the homoplasy of traits relating to ant-plant interaction such as EFNs (Marazzi & Sanderson, 2010; Weber & Agrawal, 2014) or the epiphytic habit (Chomicki *et al.*, 2015), which both show

more clustered phylogenetic patterns. A pattern first revealed here is the parallel (homoplastic) evolution of similar domatium types among closely related species, for example in the Miconieae. The frequent domatium loss that we also detected might relate to antagonistic interactions among closely related species competing for plant-ants.

Traits that may have facilitated domatium evolution

Traits that may facilitate the evolution of ant-plant symbioses have long been discussed (Benson, 1985; Davidson & McKey, 1993). One such trait might be extrafloral nectaries, in which case one would expect that many myrmecophyte lineages would ancestrally have EFNs. Our trait reconstructions, however, revealed that although 14 myrmecophyte lineages have EFNs (African *Vachellia*, *Barteria*, *Callicarpa japonica*, *Fagraea*, *Ficus obscura*, *Hirtella*, *Humboldtia*, *Leonardoxa*, *Macaranga*, *Nepenthes bicalcarata*, *Pometia*, *Shorea acuminata*, Mesoamerican *Vachellia*, *Zanthoxylum*; Fig. S17), only in Mesoamerican *Vachellia* did

domatium-bearing species clearly evolve from EFN-bearing ancestors (Fig. S17b).

Many different plant organs have been transformed into domatia (Fig. 1). Stipular thorns and large bud-protecting stipules likely facilitated the evolution of stipular domatia (Davidson & McKey, 1993). Palmate venation, found for instance in Neotropical Melastomataceae and African Malvaceae, may have fostered the evolution of leaf pouches, whether for mites or for ants (Schnell & Grout de Beaufort, 1966; O'Dowd & Willson, 1989; Davidson & McKey, 1993). Stem domatia require a minimal diameter of primary stem, and thus seem to have evolved primarily in thick-stemmed plants. Another trait that may have facilitated domatium evolution is seed dispersal by ants, and indeed Australasian epiphytic myrmecophytes (e.g. *Dischidia*, *Hoya*, *Hydnophytum*, *Myrmecodia*) often form ant gardens and are also dispersed by ants (Huxley, 1978, 1980; Kaufmann, 2002; Kaufmann & Maschwitz, 2006).

The growth form spectrum of myrmecophytes

Figure 4 shows that among ant-plants, lianas and herbs are rare growth forms and that the Neotropics have very few epiphytic myrmecophytes. Given that *c.* 19% of tropical vascular plants species are climbers and 41% are herbs (Gentry & Dodson, 1987; Gentry, 1991), the 4.5% climbers and 3.1% herbs found among ant-plants are far fewer than expected (Fig. 4). The underrepresentation of herbs may relate to the minimum plant size and generation time needed to bear and maintain domatia. In the Neotropics, all 20 herbaceous ant-plants are *Piper* species from section *Macrostachys* that form large perennial herbs. The remaining herbaceous ant plants also are perennials with sufficiently large stems (Jolivet, 1973, 1996; Champluvier, 1994; Kaufmann *et al.*, 2001; Tepe *et al.*, 2004; our Table S1). The underrepresentation of climbers may relate to their narrow stems and numerous contact points with surrounding vegetation, increasing the likelihood of invasion by nonmutualistic ants. In the African climbing ant-plant *Vitex thyrsoiflora*, occupied by the specialized ant *Tetraponera tessmanii*, morphological and behavioural filters prevent other ants from entering the domatia (Djiéto-Lordon *et al.*, 2005).

Recent ages of domatium-based symbioses compared to seed dispersal and nectar-feeding mutualisms

A major finding of this study is that ant plant lineages in Africa may not be much older than 5 Myr and those in the Neotropics and Australasia not much older than 15 Myr (Fig. 5). The younger age of African myrmecophytes, associated with a three times lower species richness than found in the other two regions (Fig. 2b), suggests that the climate oscillations in tropical Africa during the late Miocene and Pliocene (van Zinderen Bakker & Mercer, 1986; Jacobs, 2004) either limited diversification or drove older myrmecophytes to extinction. During the middle Miocene, starting from *c.* 16 Ma onwards, the African continent underwent gradual cooling and uplift in the east and south, leading to an expansion of woodlands and savannas, and reducing

lowland rain forests (van Zinderen Bakker & Mercer, 1986; Jacobs, 2004), which harbour most African myrmecophytes. The mid-Miocene ages of Neotropical *Pseudomyrmex* (Pseudomyrmecinae) and *Azteca* (Dolichoderinae) inferred in other studies (Gómez-Acevedo *et al.*, 2010; Pringle *et al.*, 2012) also fit with a relatively recent evolution of ant–plant symbioses (Fig. 5).

Because ants and plants have been coexisting for at least 120 Myr (Brady *et al.*, 2006; Bell *et al.*, 2010; Moreau & Bell, 2013), it has been suggested that ant–plant symbioses in general are old, notwithstanding a few young ant–plant symbioses (Davidson & McKey, 1993). For example, Jolivet (1996: 169) suggested that ‘Myrmecodomy must be very old, i.e. since the Cretaceous, and it is at that period that we find the first fossil ants.’ Yet none of the 46 myrmecophyte lineages for which we estimated crown group ages predates the mid-Miocene. Seed dispersal by ants (myrmecochory) apparently had arisen by 75 Ma (Dunn *et al.*, 2007; Lengyel *et al.*, 2009), whereas EFNs have arisen over the past 50 Myr (*Passiflora*: 322 species with EFNs, 40 Myr, Hearn, 2006; *Inga*: 294 species with EFNs, 10 Myr, Richardson *et al.*, 2001; *Senna*: *c.* 250 species with EFN; 40 Myr, Marazzi & Sanderson, 2010), and no EFN fossils pre-date the Oligocene (*Populus*: Pemberton, 1992; *Macaranga*, *Mallotus*: Nucete *et al.*, 2012). Today, EFNs are known from 3941 vascular plants, and they evolved at least 457 times (Weber & Keeler, 2013), whereas domatia are known from 681 vascular plants and evolved minimally 158 times (this study). Ant domatia so far have no fossil record, different from mite domatia (O'Dowd *et al.*, 1991).

From these data, the evolutionary sequence of ant–plant mutualisms may have begun with seed dispersal in the Late Cretaceous, followed by EFNs in the Eocene, and the evolution of domatium nesting during the Miocene. In their dynamic-succession hypothesis, Wilson & Hölldobler (2005) emphasized the importance of complex habitats provided by angiosperms for the transition of ants from a diet based on insect predation to harvesting hemipteran secretions and EFN nectar. Sugary secretions as a diet supplement are key to arboreal ant diets because there are not enough canopy-dwelling insects to sustain large ant colonies hunting for insect prey (Davidson *et al.*, 2003; Wilson & Hölldobler, 2005). The Miocene origin of many epiphyte domatia inferred in our study is consistent with Wilson & Hölldobler's scenario because these domatia are inhabited by arboreal ants, often tending scale insects and collecting nectar from EFNs. Because nesting space is commonly a limiting resource for ants (Philpott & Foster, 2005), the evolution of domatia as additional ant nesting sites may have been in part driven by Miocene ant radiations in tropical canopies (Brady *et al.*, 2006; Moreau *et al.*, 2006; Moreau & Bell, 2013). At the same time, canopy epiphytes clearly gain from the additional nutrients coming from symbiotic ants (Benzing, 1970; Huxley, 1978; Rickson, 1979; Rico-Gray *et al.*, 1989; Gay, 1993; Treseder *et al.*, 1995; Gegenbauer *et al.*, 2012).

Conclusions

There now are 681 known ant-plant species (Table S1), but their true number may be as high as 1139 species. The absence of the

ancestral trait 'EFN' in most domatium-bearing lineages suggests that ant-plant symbioses rarely evolved from pre-existing defence mutualisms. More commonly, they may have evolved from parasitic relationships with scale insects, a trait we did not analyse *per se*, but which is common in domatium-living ants. Among the largest ant-plant groups (all molecular-clock dated here), none are older than 19 Myr, that is early Miocene, with African ant-plant symbioses apparently not pre-dating the late Miocene. Radiations of domatium-bearing lineages have produced few sizeable clades (Hydnophytinae, *c.* 100 species, 80 of them ant-plants; *Cecropia*, 61 species, 48 ant-plants), losses of domatia are frequent, and radiations in the associated plant-ants are also species-poor, resulting in a scattered phylogenetic distribution (Fig. S16). There is thus no straightforward effect of ant-plant symbioses on diversification rates.

Acknowledgements

We thank Matthew Jebb for his unpublished revision of *Hydnophytum* and Camilla Huxley-Lambrick for an unpublished list of ant-plants; William J. Baker, Milan Janda, Kathleen Keeler, Conrad Labandeira, Marjorie Webber and Volker Witte for discussion; Brigitte Fiala, Eduard Linsenmair, Nigel Smith and Andreas Wistuba for the photographs used in Fig. 1; and Jeremy Aroles for helping compile the species list in Table S1. We thank Martin Heil, Doyle McKey and an anonymous reviewer for critical comments that helped improve the manuscript.

References

- Barrabé L, Maggia L, Pillon Y, Rigault F, Mouly A, Davis AP, Buerki S. 2014. New Caledonian lineages of *Psychotria* (Rubiaceae) reveal different evolutionary histories and the largest documented plant radiation for the archipelago. *Molecular Phylogenetics and Evolution* 71: 15–35.
- Bell CD, Soltis DE, Soltis PS. 2010. The age and diversification of the angiosperms re-revisited. *American Journal of Botany* 97: 1296–1303.
- Benson WW. 1985. Amazon ant/plants. In: Prance GT, Lovejoy TE, eds. *Amazonia*. Oxford, UK: Pergamon Press, 239–266.
- Benzing DH. 1970. An investigation of two bromeliad myrmecophytes: *Tillandsia butzii* Mez, *T. caput-medusae* E. Morren, and their ants. *Bulletin of the Torrey Botanical Club* 97: 109–115.
- Blattner FR, Weising K, Bänfer G, Maschwitz U, Fiala B. 2001. Molecular analysis of phylogenetic relationships among myrmecophytic *Macaranga* species (Euphorbiaceae). *Molecular Phylogenetics and Evolution* 19: 331–344.
- Brady SG, Schultz TR, Fisher BL, Ward PS. 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proceedings of the National Academy of Sciences, USA* 103: 18172–18177.
- Bremer B, Eriksson T. 2009. Time tree of Rubiaceae: phylogeny and dating the family, subfamilies, and tribes. *International Journal of Plant Sciences* 170: 766–793.
- Brouat C, Gielly L, McKey D. 2001. Phylogenetic relationships in the genus *Leonardoxa* (Leguminosae: Caesalpinioideae) inferred from chloroplast trnL intron and trnL-trnF intergenic spacer sequences. *American Journal of Botany* 88: 143–149.
- Brouat C, McKey D, Douzery EJP. 2004. Differentiation in a geographical mosaic of plants coevolving with ants: phylogeny of the *Leonardoxa africana* complex (Fabaceae: Caesalpinioideae) using amplified fragment length polymorphism markers. *Molecular Ecology* 13: 1157–1171.
- Bunge J. 2011. Estimating the number of species with CatchAll. *Pacific Symposium on Biocomputing* 2011: 121–130.
- Champluvier D. 1994. *Brachystephanus myrmecophilus* (Acanthaceae), espèce nouvelle du Zaïre oriental: un cas intéressant de myrmécophilie. *Belgian Journal of Botany* 127: 45–60.
- Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD, Duval MR, Price A, Hills HG, Qiu Y-L *et al.* 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. *Annals of the Missouri Botanical Garden* 80: 528–580.
- Chenuil A, McKey DB. 1996. Molecular phylogenetic study of a myrmecophyte symbiosis: did *Leonardoxa* ant associations diversify via cospeciation? *Molecular Phylogenetics and Evolution* 6: 270–286.
- Chomicki G, Bidet LPR, Ming F, Coiro M, Zhang X, Wang Y, Baissac Y, Jay-Allemand C, Renner SS. 2015. The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic. *New Phytologist* 205: 1330–1341.
- Cruaud A, Rønsted N, Chantarasuwan B, Chou LS, Clement WL, Couloux A, Savolainen V. 2012. An extreme case of plant–insect codiversification: figs and fig-pollinating wasps. *Systematic Biology* 61: 1029–1047.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.
- Davidson DW, Cook SC, Snelling RR, Chua TH. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* 300: 969–972.
- Davidson DW, McKey D. 1993. The evolutionary ecology of symbiotic ant/plant relationships. *Journal of Hymenopteran Research* 2: 13–83.
- Davies SJ, Lum SK, Chan R, Wang LK. 2001. Evolution of myrmecophytism in western Malesian *Macaranga* (Euphorbiaceae). *Evolution* 55: 1542–1559.
- Dejean A, Olmsted I, Snelling RR. 1995. Tree-epiphyte–ant relationships in the low inundated forest of Sian Ka'an Biosphere Reserve, Quintana Roo, Mexico. *Biotropica* 27: 57–70.
- Djiéto-Lordon C, Dejean A, Ring RA, Nkongmeneck BA, Lauga J, McKey D. 2005. Ecology of an improbable association: the pseudomyrmecine plant-ant *Tetraponera tessmanni* and the myrmecophytic liana *Vitex thyrsoiflora* (Lamiaceae) in Cameroon. *Biotropica* 37: 421–430.
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M. 2011. *Geneious, version 5.4*. Auckland, New Zealand: Geneious.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.
- Dunn RR, Gove AD, Barraclough TG, Givnish TJ, Majer JD. 2007. Convergent evolution of an ant-plant mutualism across plant families, continents, and time. *Evolutionary Ecology Research* 9: 1349.
- Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18: 586–608.
- Federle W, Maschwitz U, Fiala B, Riederer M, Hölldobler B. 1997. Slippery ant-plants and skilful climbers: selection and protection of specific ant partners by epicuticular wax blooms in *Macaranga* (Euphorbiaceae). *Oecologia* 112: 217–224.
- Federle W, Rohrseitz K, Hölldobler B. 2000. Attachment forces of ants measured with a centrifuge: better 'wax-runners' have a poorer attachment to a smooth surface. *Journal of Experimental Biology* 203: 505–512.
- Gay H. 1993. Animal-fed plants: an investigation into the uptake of ant-derived nutrients by the far-eastern epiphytic fern *Lecanopteris Reinw.* (Polypodiaceae). *Biological Journal of the Linnean Society* 50: 221–233.
- Gegenbauer C, Mayer VE, Zotz G, Richter A. 2012. Uptake of ant-derived nitrogen in the myrmecophytic orchid *Caularthron bilamellatum*. *Annals of Botany* 110: 757–766.
- Gentry AH. 1991. The distribution and evolution of climbing plants. In: Putz FE, Mooney HA, eds. *The biology of vines*. Cambridge, UK: Cambridge University Press.
- Gentry AH, Dodson CH. 1987. Diversity and biogeography of neotropical vascular epiphytes. *Annals of the Missouri Botanical Garden* 74: 205–233.
- Gómez-Acevedo S, Rico-Arce L, Delgado-Salinas A, Magallón S, Eguarte LE. 2010. Neotropical mutualism between *Acacia* and *Pseudomyrmex*: phylogeny and divergence times. *Molecular Phylogenetics and Evolution* 56: 393–408.

- Gottsclling M, Mai DH, Hilger HH. 2002. The systematic position of *Ehretia* fossils (Ehretiaceae, Boraginales) from the European Tertiary and implications for character evolution. *Review of Palaeobotany and Palynology* 121: 149–156.
- Hearn DJ. 2006. *Adenia* (Passifloraceae) and its adaptive radiation: phylogeny and growth form diversification. *Systematic Botany* 31: 805–821.
- Heil M, González-Teuber M, Clement LW, Kautz S, Verhaagh M, Bueno JCS. 2009. Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proceedings of the National Academy of Sciences, USA* 106: 18091–18096.
- Heil M, Rattke J, Boland W. 2005. Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* 308: 560–563.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.
- Huxley CR. 1978. The ant-plants Myrmecodia and Hydnophytum Rubiaceae, and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytologist* 80: 231–268.
- Huxley CR. 1980. Symbiosis between ants and epiphytes. *Biological Reviews* 55: 321–340.
- Jacobs BF. 2004. Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions of the Royal Society B* 359: 1573–1583.
- Janzen DH. 1967. Interaction of the bull's-horn acacia (*Acacia cornigera* L.) with an ant inhabitant (*Pseudomyrmex ferruginea* F. Smith) in eastern Mexico. *University of Kansas Science Bulletin* 47: 315–558.
- Janzen DH. 1969. Allelopathy by myrmecophytes: the ant *Azteca* as an allelopathic agent of *Cecropia*. *Ecology* 50: 147–153.
- Janzen DH. 1974. Epiphytic myrmecophytes in Sarawak: mutualism through the feeding of plants by ants. *Biotropica* 6: 237–259.
- Jolivet P. 1973. Les plantes myrmecophiles du Sud Est Asiatique. *Cahiers du Pacifique* 17: 41–69.
- Jolivet P. 1996. *Ants and plants: an example of coevolution*. Leiden, the Netherlands: Backhuys.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
- Kaufmann E. 2002. *Southeast Asian ant-gardens: diversity, ecology, ecosystematic significance, and evolution of mutualistic ant–epiphyte associations*. PhD thesis, University of Frankfurt, Frankfurt, Germany.
- Kaufmann E, Maschwitz U. 2006. Ant-gardens of tropical Asian rainforests. *Naturwissenschaften* 93: 216–227.
- Kaufmann E, Weissflog A, Hashim R, Maschwitz U. 2001. Ant-gardens on the giant bamboo *Gigantochloa scorteichinii* (Poaceae) in West-Malaysia. *Insectes Sociaux* 48: 125–133.
- Kay KM, Whittall JB, Hodges SA. 2006. A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. *BMC Evolutionary Biology* 6: 36.
- Keeler KH. 2008. *World list of angiosperms with extrafloral nectaries*. [WWW document] URL <http://biosci-labs.unl.edu/Emeriti/keeler/extrafloral/worldlistfamilies.htm> [accessed 1 June 2014].
- Lavin M, Herendeen PS, Wojciechowski MF. 2005. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. *Systematic Biology* 54: 575–594.
- Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR. 2009. Ants sow the seeds of global diversification in flowering plants. *PLoS ONE* 4: e5480.
- Lewis PO. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50: 913–925.
- Maddison WP, Maddison DR. 2011. *Mesquite 2.75: a modular system for evolutionary analysis*. [WWW document] URL <http://mesquiteproject.org> [accessed 1 June 2014].
- Magallón S, Hilu KW, Quandt D. 2013. Land plant evolutionary timeline: gene effects are secondary to fossil constraints in relaxed clock estimation of age and substitution rates. *American Journal of Botany* 100: 556–573.
- Manen JF, Natali A. 1995. Comparison of the evolution of ribulose-1, 5-biphosphate carboxylase (rbcL) and atpB-rbcL noncoding spacer sequences in a recent plant group, the tribe Rubieae (Rubiaceae). *Journal of Molecular Evolution* 41: 920–927.
- Marazzi B, Sanderson MJ. 2010. Large-scale patterns of diversification in the widespread legume genus *Senna* and the evolutionary role of extrafloral nectaries. *Evolution* 64: 3570–3592.
- Markstädter C, Federle W, Jetter R, Riederer M, Hölldobler B. 2000. Chemical composition of the slippery epicuticular wax blooms on *Macaranga* (Euphorbiaceae) ant-plants. *Chemoecology* 10: 33–40.
- Mayer VE, Frederickson ME, McKey D, Blatrix R. 2014. Current issues in the evolutionary ecology of ant–plant symbioses. *New Phytologist* 202: 749–764.
- McKey D, Davidson DW. 1993. Ant–plant symbioses in Africa and the Neotropics: history, biogeography and diversity. In: Goldblatt P, ed. *Biological relationships between Africa and South America*. Yale, CT, USA: Yale University Press, 568–606.
- Michelangeli FA, Penneys DS, Giza J, Soltis D, Hils MH, Skee JD. 2004. A preliminary phylogeny of the tribe Miconieae (Melastomataceae) based on nrITS sequence data and its implications on inflorescence position. *Taxon* 53: 279.
- Moreau CS, Bell CD. 2013. Testing the ‘museum versus cradle’ tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. *Evolution* 67: 2240–2257.
- Moreau CS, Bell CD, Vila R, Archibald SB, Pierce NE. 2006. Phylogeny of the ants: diversification in the age of angiosperms. *Science* 312: 101–104.
- Nucete M, Van Konijnenburg-van Cittert JHA, Van Welzen PC. 2012. Fossils and palaeontological distributions of *Macaranga* and *Mallotus* (Euphorbiaceae). *Palaeogeography, Palaeoclimatology, Palaeoecology* 353: 104–115.
- O'Dowd DJ, Brew CR, Christophel DC, Norton RA. 1991. Mite-plant associations from the Eocene of southern Australia. *Science* 252: 99–101.
- O'Dowd DJ, Willson MF. 1989. Leaf domatia and mites on Australasian plants: ecological and evolutionary implications. *Biological Journal of the Linnean Society* 37: 191–236.
- Orona-Tamayo D, Heil M. 2013. Stabilizing mutualisms threatened by exploiters: new insights from ant–plant research. *Biotropica* 45: 654–665.
- Pagel M, Meade A. 2007. *BayesTraits*. Computer program and documentation. [WWW document] URL <http://www.evolution.rdg.ac.uk/BayesTraits.html> [accessed 1 June 2014].
- Peccoud J, Piatscheck F, Yockteng R, Garcia M, Sauve M, Djiéto-Lordon C, Harris DJ, Blatrix R. 2013. Multi-locus phylogenies of the genus *Barteria* (Passifloraceae) portray complex patterns in the evolution of myrmecophytism. *Molecular Phylogenetics and Evolution* 66: 824–832.
- Pemberton RW. 1992. Fossil extrafloral nectaries, evidence for the ant-guard antitherbivore defense in an Oligocene *Populus*. *American Journal of Botany* 79: 1242–1246.
- Philpott SM, Foster PF. 2005. Nest-site limitation in coffee agroecosystems: artificial nests maintain diversity of arboreal ants. *Ecological Applications* 15: 1478–1485.
- Pringle EG, Ramirez SR, Bonebrake TC, Gordon DM, Dirzo R. 2012. Diversification and phylogeographic structure in widespread Azteca plant-ants from the northern Neotropics. *Molecular Ecology* 21: 3576–3592.
- Quek SP, Davies SJ, Itino T, Pierce NE. 2004. Codiversification in an ant–plant mutualism: stem texture and the evolution of host use in Crematogaster (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). *Evolution* 58: 554–570.
- Rambaut A, Drummond AJ. 2009. *Tracer v1.5*. Edinburgh (UK). [WWW document] URL <http://tree.bio.ed.ac.uk/software/tracer/> [accessed 1 June 2014].
- Razafimandimbison SG, Moog J, Lantz H, Maschwitz U, Bremer B. 2005. Re-assessment of monophyly, evolution of myrmecophytism, and rapid radiation in *Neonauclea* s.s. (Rubiaceae). *Molecular Phylogenetics and Evolution* 34: 334–354.
- Renner SS. 1997. *Tococa caryophyllaea* (DC.) Renner (Melastomataceae): a climbing *Tococa*. *BiolLania* 6 (Wurdack Festschrift): 497–500.
- Renner SS, Ricklefs RE. 1998. Herbicidal activity of domatia-inhabiting ants in patches of *Tococa guianensis* and *Clidemia heterophylla*. *Biotropica* 30: 324–327.
- Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* 293: 2242–2245.

- Rickson FR. 1979. Absorption of animal tissue breakdown products into a plant stem—the feeding of a plant by ants. *American Journal of Botany* **66**: 87–90.
- Rico-Gray V, Barber JT, Thien LB, Ellgaard EG, Toney JJ. 1989. An unusual animal-plant interaction: feeding of *Schomburgkia tibicinis* (Orchidaceae) by ants. *American Journal of Botany* **76**: 603–608.
- Sanchez A, Kron KA. 2008. Phylogenetics of Polygonaceae with an emphasis on the evolution of Eriogonoideae. *Systematic Botany* **33**: 87–96.
- Saslis-Lagoudakis C, Chase MW, Robinson DN, Russell SJ, Klitgaard BB. 2008. Phylogenetics of neotropical *Platymiscium* (Leguminosae: Dalbergiaceae): systematics, divergence times, and biogeography inferred from nuclear ribosomal and plastid DNA sequence data. *American Journal of Botany* **95**: 1270–1286.
- Schnell R, Grout de Beaufort F. 1966. Contribution à l'étude des plantes à myrmécodomaties de l'Afrique intertropicale. *Mémoire de l'Institut Fondamental d'Afrique Noire* **75**: 1–66.
- Schuster TM, Setaro SD, Kron KA. 2013. Age estimates for the buckwheat family Polygonaceae based on sequence data calibrated by fossils and with a focus on the Amphi-Pacific *Muehlenbeckia*. *PLoS ONE* **8**: e61261.
- Smith SA, Donoghue MJ. 2008. Rates of molecular evolution are linked to life history in flowering plants. *Science* **322**: 86–89.
- Smith JF, Stevens AC, Tepe EJ, Davidson C. 2008. Placing the origin of two species-rich genera in the late cretaceous with later species divergence in the tertiary: a phylogenetic, biogeographic and molecular dating analysis of *Piper* and *Peperomia* (Piperaceae). *Plant Systematics and Evolution* **275**: 9–30.
- Smith SA, Dunn CW. 2008. Phyutility: a phyloinformatics tool for trees, alignments and molecular data. *Bioinformatics* **24**: 715–716.
- Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**: 758–771.
- Tepe EJ, Vincent MA, Watson LE. 2004. Phylogenetic patterns, evolutionary trends, and the origin of ant-plant associations in *Piper* section *Macrostachys*: Burger's hypotheses revisited. In: Dayer LA, Palmer AND, eds. *Piper: a model genus for studies of phytochemistry, ecology, and evolution*. New York, NY, USA: Springer, 156–178.
- Treseder KK, Davidson DW, Ehleringer JR. 1995. Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature* **375**: 137–139.
- de Vienne DM, Refrégier G, López-Villavicencio M, Tellier A, Hood ME, Giraud T. 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist* **198**: 347–385.
- Wanntorp L, Kocyan A, Renner SS. 2006. Wax plants disentangled: a phylogeny of *Hoya* (Marsdeniaceae, Apocynaceae) inferred from nuclear and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* **39**: 722–733.
- Ward PS. 1991. Phylogenetic analysis of pseudomyrmecine ants associated with domatia-bearing plants. In: Huxley CR, ed. *Ant-plant interactions*. Oxford, UK: Oxford University Press, 335–352.
- Ward PS, Downie DA. 2005. The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. *Systematic Entomology* **30**: 310–335.
- Weber MG, Agrawal AA. 2014. Defense mutualisms enhance plant diversification. *Proceedings of the National Academy of Sciences, USA* **111**: 16442–16447.
- Weber MG, Keeler KH. 2013. The phylogenetic distribution of extrafloral nectaries in plants. *Annals of Botany* **111**: 1251–1261.
- Weeks A, Baird KE, McMullen CK. 2010. Origin and evolution of endemic Galápagos *Varronia* species (Cordiaceae). *Molecular Phylogenetics and Evolution* **57**: 948–954.
- Weigend M, Luebert F, Gottschling M, Couvreur TL, Hilger HH, Miller JS. 2013. From capsules to nutlets—phylogenetic relationships in the Boraginales. *Cladistics* **30**: 508–518.
- van Welzen PC, Strijk JS, van Konijnenburg-van Cittert JH, Nucete M, Merckx VS. 2014. Dated phylogenies of the sister genera *Macaranga* and *Mallotus* (Euphorbiaceae): congruence in historical biogeographic patterns? *PLoS ONE* **9**: e85713.
- Wilson EO, Hölldobler B. 2005. The rise of the ants: a phylogenetic and ecological explanation. *Proceedings of the National Academy of Sciences, USA* **102**: 7411–7414.
- van Zinderen Bakker EM, Mercer JH. 1986. Major late Cenozoic climatic events and palaeoenvironmental changes in Africa viewed in a world wide context. *Palaeogeography, Palaeoclimatology, Palaeoecology* **56**: 217–235.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 1181-species tree from Fig. 3 with species names.

Fig. S2 Dated phylogeny of *Barteria* (Passifloraceae).

Fig. S3 Dated phylogeny of *Cecropia* (Urticaceae).

Fig. S4 Dated phylogeny of *Clerodendrum* (Lamiaceae).

Fig. S5 Dated phylogeny of *Cordia* (Cordiaceae).

Fig. S6 Dated phylogeny of *Dischidia* (Apocynaceae).

Fig. S7 Dated phylogeny of the Hydnophytinae (*Anthorrhiza*, *Hydnophytum*, *Myrmecodia*, *Myrmephytum*, *Squamellaria*; Rubiaceae).

Fig. S8 Dated phylogeny of *Leonardoxa* (Fabaceae – Caesalpinioideae).

Fig. S9 Dated phylogeny of *Macaranga* (Euphorbiaceae).

Fig. S10 Dated phylogeny of the Miconieae (*Clidemia*, *Maieta*, *Tococa* and *Topobea* (the latter, Blakeaceae); Melastomataceae).

Fig. S11 Dated phylogeny of *Nauclea* and *Neonauclea* (Rubiaceae).

Fig. S12 Dated phylogeny of *Piper* (Piperaceae).

Fig. S13 Dated phylogeny of *Platymiscium* (Fabaceae – Faboideae).

Fig. S14 Dated phylogeny of *Ruprechtia* and *Triplaris* (Polygonaceae).

Fig. S15 Dated phylogeny of *Vachellia* (Fabaceae – Mimosoideae).

Fig. S16 Number of plant-ants per genus in the six subfamilies containing plant-ants.

Fig. S17 Comparison of domatium and EFN evolution on the 1181 taxa tree.

Table S1 The World list of ant domatium-bearing plants

Table S2 Genbank accession numbers for phylogenies generated in this study (Figs 1, S1–S15)

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