

UC Davis

UC Davis Previously Published Works

Title

Macroevolutionary assembly of ant/plant symbioses: Pseudomyrmex ants and their ant-housing plants in the Neotropics

Permalink

<https://escholarship.org/uc/item/49r6h9s2>

Journal

Proceedings of the Royal Society B, 282(1819)

ISSN

0962-8452

Authors

Chomicki, Guillaume

Ward, Philip S

Renner, Susanne S

Publication Date

2015-11-22

DOI

10.1098/rspb.2015.2200

Peer reviewed



Research

Cite this article: Chomicki G, Ward PS, Renner SS. 2015 Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. *Proc. R. Soc. B* **282**: 20152200. <http://dx.doi.org/10.1098/rsob.2015.2200>

Received: 12 September 2015
Accepted: 26 October 2015

Subject Areas:
evolution, ecology

Keywords:
ant/plant coevolution, co-radiation,
secondary colonization, molecular clocks,
Pseudomyrmex, symbiosis

Author for correspondence:
Guillaume Chomicki
e-mail: guillaume.chomicki@gmail.com

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsob.2015.2200> or via <http://rsob.royalsocietypublishing.org>.

Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics

Guillaume Chomicki¹, Philip S. Ward² and Susanne S. Renner¹

¹Systematic Botany and Mycology, Department of Biology, University of Munich (LMU), 80638 Munich, Germany
²Department of Entomology and Nematology, University of California, Davis, CA 95616, USA

Symbioses include some of the clearest cases of coevolution, but their origin, loss or reassembly with different partners can rarely be inferred. Here we use ant/plant symbioses involving three plant clades to investigate the evolution of symbioses. We generated phylogenies for the big-eyed arboreal ants (*Pseudomyrmecinae*), including 72% of their 286 species, as well as for five of their plant host groups, in each case sampling more than 61% of the species. We show that the ant-housing *Vachellia* (*Mimosoideae*) clade and its ants co-diversified for the past 5 Ma, with some species additionally colonized by younger plant-nesting ant species, some parasitic. An apparent co-radiation of ants and *Tachigali* (*Caesalpinioideae*) was followed by waves of colonization by the same ant clade, and subsequent occupation by a younger ant group. Wide crown and stem age differences between the ant-housing genus *Triplaris* (*Polygonaceae*) and its obligate ant inhabitants, and stochastic trait mapping, indicate that its domatium evolved earlier than the ants now occupying it, suggesting previous symbioses that dissolved. Parasitic ant species evolved from generalists, not from mutualists, and are younger than the mutualistic systems they parasitize. Our study illuminates the macroevolutionary assembly of ant/plant symbioses, which has been highly dynamic, even in very specialized systems.

1. Introduction

The origin, maintenance, and breakdown of mutualisms are key questions in ecology and evolutionary biology [1–3]. Mapping traits of the mutualists and non-mutualist relatives on time-calibrated phylogenies has proved a powerful approach to unveil the temporal and geographical origin of mutualisms. A finding of co-phylogenetic studies of mutualisms is that co-speciation is rare (reviewed in [4]) and restricted to a few symbioses, especially those with vertical transmission, such as *Buchnera* bacterial endosymbionts and aphids [5–7]. Co-speciation in mutualistic partnerships that do not involve vertical transmission may exist in some obligate systems—for instance figs and their wasp pollinators as suggested by matching divergence times, although occasional wasp switches to other figs have been documented [8]. Other obligate mutualisms, such as the *Yucca*/yucca moth pollination mutualism, were found to have evolved multiple times [9,10]. Non-specialized mutualisms can exist between partners of highly discordant ages, for example, between introduced plants and native insect or bird pollinators [11]. Only species-dense molecular clock-dated phylogenies of both partner lineages therefore can elucidate the evolution of mutualistic systems. Such analyses over the past few years have revealed that cheaters rarely evolve from mutualists, contrary to theory [3].

Ant/plant symbioses involve plants with modified structures (domatia) that house ants, in return for protection or extra nutrients and sometimes also the physical or chemical removal of competing plant species [12–14]. Ant/plant symbioses appear to be younger than seed dispersal by ants or extrafloral nectary-mediated plant defence by ants, with no extant domatium-bearing clade older than 20 Myr [14]. Few co-phylogenetic studies of ant/plant systems

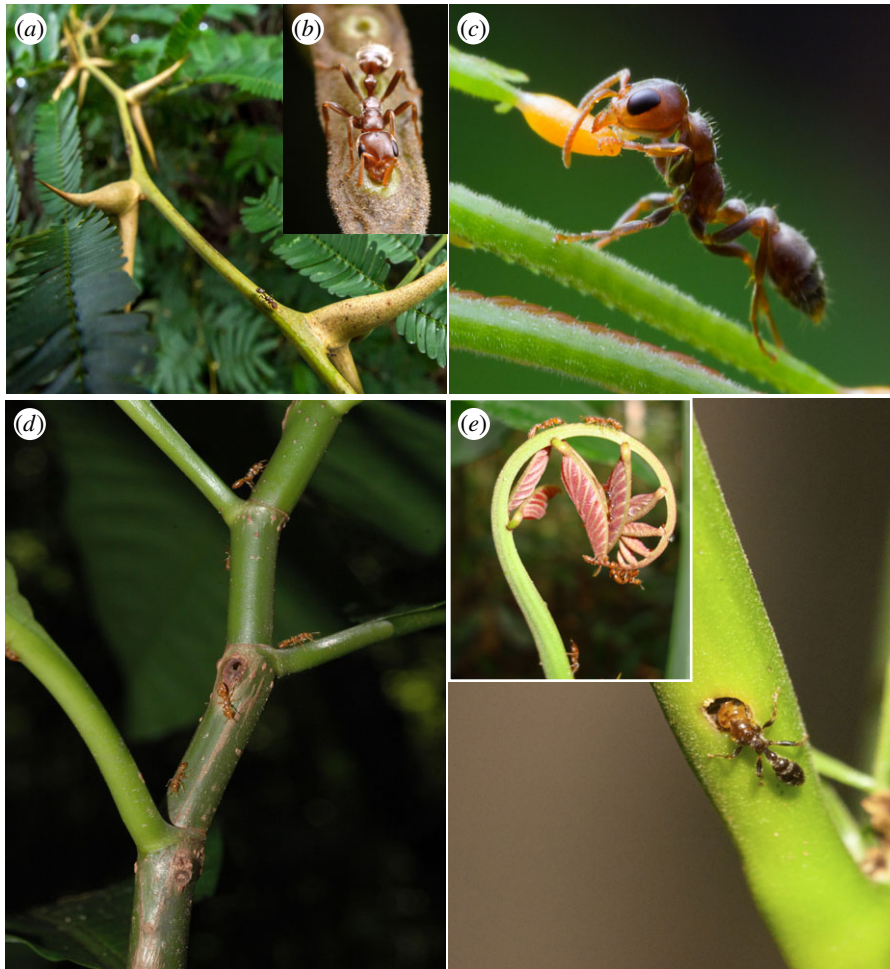


Figure 1. Examples of *Pseudomyrmex*/plant symbioses. (a–c) *Vachellia*/*Pseudomyrmex peperi* symbiosis. (a) *Vachellia* habit with stipular thorn domatia. (b) *Pseudomyrmex peperi* worker feeding on the large *Vachellia* extrafloral nectaries. (c) *Pseudomyrmex peperi* collecting a protein-rich Beltian body from the *Vachellia* leaflet tips. (d) *Triplaris americana* domatium inhabited by *Pseudomyrmex triplarinus*. (e) *Tachigali myrmecophila*/*Pseudomyrmex concolor*-group symbiosis. (e, inset) *P. concolor* entering in a *Tachigali myrmecophila* leaf domatium. (e) *Pseudomyrmex penetrator* entering the leaf rachis domatium, where an entrance hole has been chewed. Photo credits: (a–c) Alexander Wild, (d) Fabian Michelangeli, (e, inset), Heraldo Vasconcelos, (e) Ricardo Solar. (Online version in colour.)

have been conducted. In the African *Leonardoxa africana*, two of four subspecies have specialized domatia that were colonized in parallel by pre-adapted ant species [15,16]. Species of the Southeast Asian *Crematogaster borneensis*-group (former subgenus *Decacrema*) independently colonized three species groups of *Macaranga*, with an apparent matching of plant stem morphology and associated ant behaviour [17]. Co-radiation has been inferred in *Pseudomyrmex* and Mesoamerican *Vachellia* [18].

Pseudomyrmecinae comprise 230 described species in three genera [19–22], with 32 of the species living in plant domatia [14,19], making *Pseudomyrmecinae* the most diverse plant-occupying ant group worldwide [14]. Of the three genera, *Myrcidris* includes two species (one undescribed) from northern South America, *Pseudomyrmex* has 134 species, also confined to the New World, and *Tetraoponera* comprises 95 species in Africa and Australasia [23]. Most species nest in dead hollow twigs of living plants, others nest only in the domatia of particular species that they protect against herbivores (figure 1), and some are parasites of other ant/plant symbioses [19,21,24,25]. Obligate domatium-nesting big-eyed ants have entered into more or less tight symbioses with species of the Fabaceae genera *Vachellia*, *Tachigali* and *Platymiscium*, and the Polygonaceae genera *Triplaris* and *Ruprechtia* [18,20,26,27]. This system is therefore ideal to study the evolution of ant/plant symbioses.

We had three expectations concerning the evolution of big-eyed ant/plant symbioses: (i) co-radiation (co-diversification) would be seen only in relatively young clades because of the increasing probability of partner loss over time, (ii) non-mutualistic domatium-nesting big-eyed ant species (i.e. parasites of existing symbioses) would be younger than mutualistic species, and (iii) highly age-discrepant partners would be rare in specialized symbioses. To evaluate geographical range shifts in both partners, we rely on a statistical biogeographic approach that allows comparing models with and without the assumption of speciation-with-dispersal [28,29]. With respect to geographical evolution, we expected that for specialized symbioses, ancestral areas of plant–ant clades should match those of their plant hosts.

2. Material and methods

(a) Taxon sampling, DNA isolation and amplification

The most important myrmecophyte genera associated with *Pseudomyrmex* ants are: *Vachellia* (Fabaceae: Mimosoideae), *Platymiscium* (Fabaceae: Faboideae), *Tachigali* (Fabaceae: Caesalpinoideae), *Triplaris* (Polygonaceae: Eriogonoideae) and *Ruprechtia*, the latter two being sister groups [30]. Our plant sampling ranged from 61 to 75% (see the electronic supplementary material, Material and Methods for details).

We sampled 64% of Pseudomyrmecinae including 78% of *Pseudomyrmex* species. Ten non-pseudomyrmecine ant species, including representatives of the sister-group (Myrmeciinae), were used as outgroups. Building on previous studies [22], we compiled or newly generated sequences from 10 nuclear markers, namely 28S rRNA, Wg, AbdA, LW Rh, EF1 α F2, ArgK, Enolase, CAD, Top1 and Ubx. Out of 2150 sequences in the Pseudomyrmecinae matrix, 1990 are new (GenBank accession no. KR828817–KR830806). Taxon names, permanent voucher numbers with linked geographical information, and GenBank accession numbers are listed in the electronic supplementary material, table S1. The aligned data matrix for Pseudomyrmecinae has been deposited in TreeBase (study accession S17550). Primer sequences are given in the electronic supplementary material, table S2.

For *Vachellia*, *Platymiscium* and the *Triplaris/Ruprechtia* clades, we used sequences from published studies [18,30,31]; markers and alignment length are described in the electronic supplementary material, Material and Methods. For *Tachigali*, we sequenced ITS1 (nuclear) and *matK*, *trnL* intron, *trnL-trnF* and *trnH-PsbA* spacers (plastid) for 36 specimens. DNA isolation, purification and amplification followed standard methods [32]. Taxon names, vouchers, geographical information and GenBank accession numbers are listed in the electronic supplementary material, tables S3 (*Vachellia*), S4 (*Platymiscium*), S5 (*Triplaris/Ruprechtia*) and S6 (*Tachigali*). For more details see the electronic supplementary material, Materials and methods.

(b) DNA sequence alignment and phylogenetic analyses

Sequence alignments were performed in MAFFT v. 7 [33] (plants) or CLUSTAL X v. 2.1 [34] (Pseudomyrmecinae), manually edited and concatenated in MESQUITE v. 2.75 [35] (plants) or MacClade v. 4.08 [36] (Pseudomyrmecinae). Maximum-Likelihood tree inference relied on RAxML v. 8.1 [37] (plants) or GARLI v. 2.0 [38] (Pseudomyrmecinae), with 100 ML bootstrap replicates. For all plant analyses, we used the GTR + Γ substitution model in RAxML, while Pseudomyrmecinae were analysed using the partition scheme identified by PartitionFinder [39] (electronic supplementary material, table S7). For *Tachigali* and the Pseudomyrmecinae, we also conducted Bayesian analyses in MRBAYES v. 3.2 [40], with partitioning by gene region for *Tachigali*, using the best-fitting models identified by jModelTest2 [41], and using the scheme identified by PartitionFinder for the Pseudomyrmecinae (electronic supplementary material, table S7). Further details are provided in the electronic supplementary material, Materials and Methods.

(c) Molecular clock dating

Molecular clock dating relied on BEAST v. 2 [42] and the GTR + Γ substitution model with empirical nucleotide frequencies and six rate categories. In all cases, we used the uncorrelated lognormal relaxed clock model, since its standard deviation was always more than 0.5. We used Yule tree priors, with Markov chain Monte Carlo (MCMC) chain lengths between 20 and 60 million generations, sampling every 10 000th generation with chain length depending on convergence as determined by examining the log files in TRACER v. 1.5 [43] after removal of an initial burn-in proportion of 10% of the trees. Fossil and secondary calibrations for all five DNA matrices are explained in detail in the electronic supplementary material, Material and methods.

(d) Ancestral state reconstructions

We coded Pseudomyrmecinae as (0) 'ground nesting', for species nesting in the ground; (1) 'arboreal generalist', for unspecialized arboreal species nesting in dead twigs or branches of various plants, but not usually in domatia; (2) 'domatium mutualist', for plant-ants nesting obligately in domatia and presenting aggressive behaviour, and (3) 'domatium parasite', for species obligately living in domatia but with a timid behaviour that results in the

absence of defense payback to their host. Species assignments to these categories are based on published studies [19,20,27,44–47] and personal observations by P.S.W. over the past 30 years. To infer the ancestral states of nesting habits, we used the ML approach implemented in MESQUITE v. 2.75 [35] with the MK1 model and the R package Ape (Ace function) [48], using as input trees both the maximum clade credibility tree from BEAST and the best ML tree from GARLI. To take into account topological uncertainties, we used two approaches: we ran MK1 reconstructions on a sample of 1000 Bayesian trees from the BEAST MCMC runs, and we used the Bayesian reversible jump MCMC approach in BayesTraits [49], which allows transition rates between character states to vary. 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, and reconstructed the nodes of interest using the command 'addnode'.

To reconstruct the evolutionary gains and losses of domatia in *Vachellia*, *Tachigali*, *Triplaris/Ruprechtia* and *Platymiscium*, we coded each tip for domatium absence (0) or domatium presence (1), using the world list of domatium-bearing plants [14]. We performed ancestral state reconstructions using the same approaches as for the Pseudomyrmecinae. These reconstructions, including the assumptions of the model used, are described further in the electronic supplementary material, Material and methods.

(e) Historical biogeography and range sizes

We coded the geographical ranges of all Pseudomyrmecinae and of all plant species in the phylogenies as: A = USA, B = Mexico to Panama including the Caribbean, C = Northern and Central Andes (Venezuela, Colombia, Ecuador, Peru, Bolivia), D = Brazil and the Guianas, E = Chile, Argentina, Paraguay and Uruguay, F = Afrotropics and G = India, southeast Asia and Australia. The coded Neotropical regions are shown in figure 2. To infer whether (i) ancestral areas of Pseudomyrmecinae clades match those of their plant hosts and whether (ii) our focal symbioses coincide with geographical range shifts, we used ancestral range reconstruction (back to 33.7 Ma) using the multimodel approach implemented in the R package BioGeoBEARS [28,29] on the BEAST chronograms.

To determine whether increased *Pseudomyrmex* specialization (here obligate nesting in a particular plant species) coincides with range narrowing or broadening, we evaluated the range size of each plant-ant species and compared it to that of its sister group based on occurrence data from a database of vouchered material compiled by P.S.W. (electronic supplementary material, table S8). We calculated range sizes as the extent of occurrence using the software DIVA-GIS [50], following an approximate minimum convex polygon. Given the dense geographical sampling of Pseudomyrmecinae (electronic supplementary material, table S8), this approach reduces the risk of overestimating range sizes. Range size calculation and sister group taxonomic composition are described in detail in the electronic supplementary material, Material and methods.

(f) Interactions

We searched the literature to obtain information about the types of interactions between the plant and ant species sampled in our study. Data for *Triplaris* and the *Pseudomyrmex triplarinus* group come mainly from [51], those for *Vachellia* and the *P. ferrugineus* group from [20] (summarized in fig. 73) and [18]. Apart from these two groups, species-level information is scarce since botanists at best note the ant genus and entomologists the plant genus. We thus included indirect data from morphological traits and notes on genera (without species names), as long as there was a geographical overlap. All inferred links are depicted as dotted lines in the respective figures.

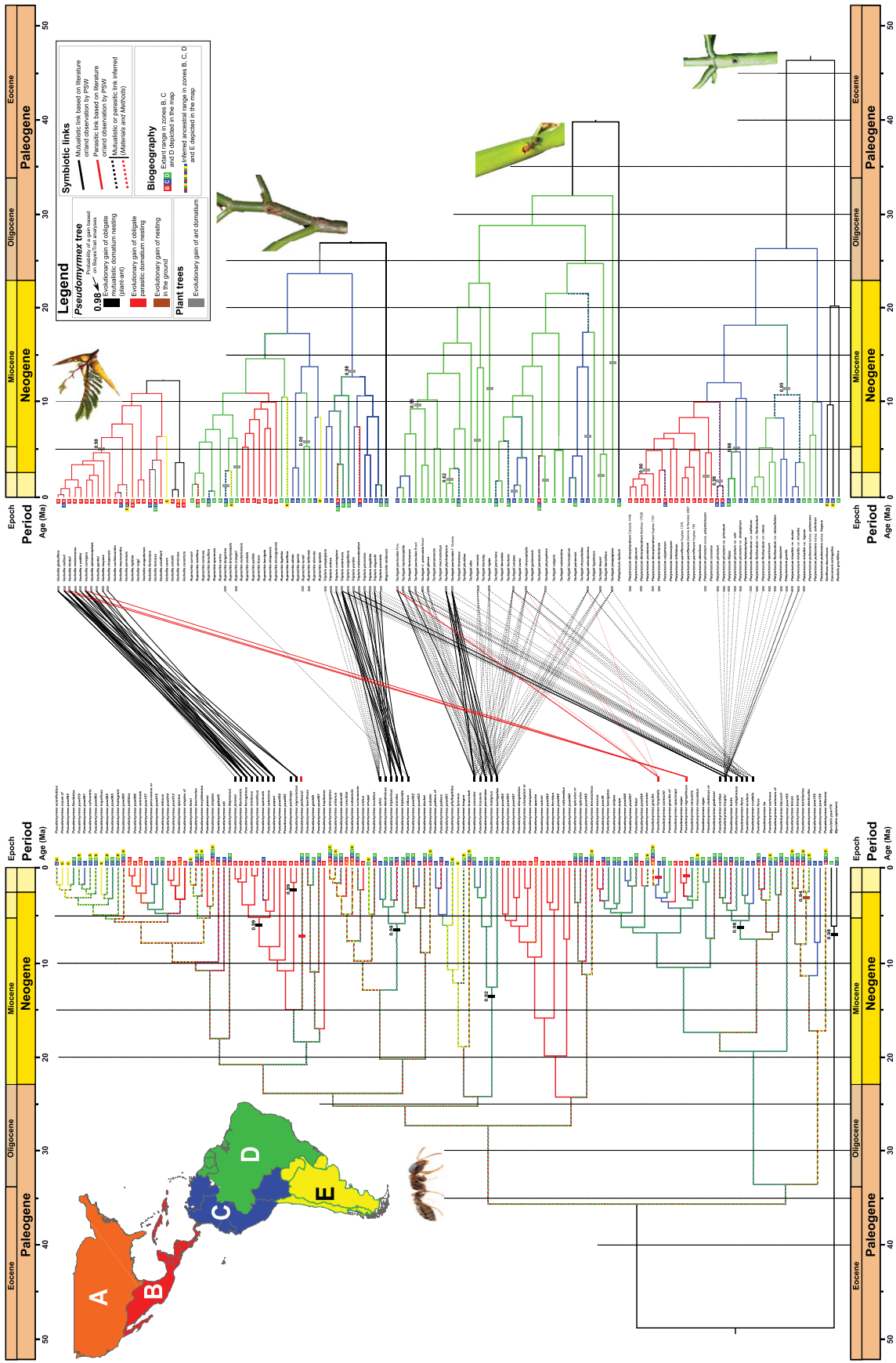


Figure 2. Dated phylogenies of *Pseudomyrmex* and its five main plant host groups: *Vachellia*, *Triplaris/Ruprechtia*, *Tachigali* and *Platymiscium*. Colour coding (map) shows the ancestral range resulting from the best-fit model (S2). Links between ant and plant species are solid black for documented mutualistic interactions, red for documented parasitic interactions, and dotted for inferred interactions. Black rectangles mark the evolutionary gain of mutualistic obligate plant nesting; red rectangles mark parasitic obligate plant nesting; and brown rectangles indicate ground nesting. The remaining *Pseudomyrmex* species are generalist arboreal ants. Grey rectangles mark the evolutionary gain of ant domesticity. Numbers above rectangles refer to the probability of an inferred gain based on BayesTraits analyses (this cannot be inferred for single species; see S2). A rectangle positioned next to a crown group means that the trait originated at that node, while the rectangle position for branches leading to single species is arbitrary. Ancestral state reconstructions are shown in the electronic supplementary material, figures S5–S10 and S12. (Online version in colour.)

3. Results

(a) Phylogenetics of Neotropical Pseudomyrmecinae and their plant hosts

Both ML and Bayesian phylogenetic inference showed a well-supported Neotropical *Myrmecodrombus* + *Pseudomyrmex* clade and four maximally supported *Pseudomyrmex* plant-ant groups (*P. ferrugineus* group, *P. concolor* group, *P. triplarinus* group and *P. sericeus* group; electronic supplementary material, figures S1 and S2). An unexpected result is that the *Vachellia* ('ant-*Acacia*') ants are not monophyletic, but instead form two clades separated by two species of arboreal generalists from Central America (figure 2), extending a previous finding [47].

Phylogenetic relationships in *Triplaris/Ruprechtia*, *Platymiscium* and *Vachellia* are as found in previous studies [18,30,31]. The monophyly of the newly investigated genus *Tachigali* is maximally supported in ML and Bayesian analyses (electronic supplementary material, figure S3), and the sister species relationships involving the position of myrmecophytes relevant to this study are well to moderately supported (electronic supplementary material, figure S3).

(b) Times of origin of Pseudomyrmecinae and their plant hosts

The most recent common ancestor (MRCA) of Pseudomyrmecinae dates to 71.7 ± 7 Ma, significantly older than found in chronograms that focused on all ants and therefore included only a few Pseudomyrmecinae [52,53]. The stem age of *Pseudomyrmex* is 49.0 ± 4 Ma, its crown age 35.8 ± 4 Ma (figure 2; electronic supplementary material, figure S4). The main clade of *Vachellia*-inhabiting species in the *P. ferrugineus* species group—here referred to as the *P. ferrugineus* subgroup—dates to 5.1 ± 1.5 Ma, matching the age of the MRCA of the Mesoamerican *Vachellia* clade, 4.7 ± 2 Ma. Two related *Vachellia*-inhabiting *Pseudomyrmex* species, *P. nigrocinctus* and *P. particeps*, forming the *P. nigrocinctus* subgroup, however, evolved 1.5 ± 1 Ma, after the radiation of the *Vachellia* species that they currently inhabit (figures 2 and 3a). Similarly, the *P. triplarinus* group evolved 5.7 ± 2 Ma, after the radiation of its obligate host clade, *Triplaris* (18 species, 61% sampled), here dated to 13 ± 2 Ma (figures 2 and 3c). The *P. concolor* species group dates to 12.2 ± 3 Ma, overlapping the age range inferred for the *Tachigali* clade that it inhabits (the *T. paniculata* group, 9.3 ± 5 Ma; figures 2 and 3b). Other ant-housing *Tachigali* species originated between 9.3 and 1.5 Ma, but the origin of domatia in single species cannot be dated (figure 2). Lastly, the *P. fortis* subgroup, a clade of myrmecophyte-inhabiting species within the *P. sericeus* species group, whose species nest in *Tachigali*, *Triplaris*, *Platymiscium* and other ant-plants, dates to 5.5 ± 1.5 Ma, and does not show any obvious crown matching with any of its hosts. Within this clade is a subgroup of strict *Tachigali* specialists, the *P. crudelis* complex, originating 3.7 ± 1 Ma, well after the *P. concolor* group.

(c) Biogeography of plant-nesting *Pseudomyrmex* and their plant hosts

BioGeoBEARS model comparison yielded the BAYAREA + J model as best fitting the ant data, significantly better than DEC + J (Lnl = -451.87 versus -608.91; electronic

supplementary material, table S9a shows all statistics of BioGeoBEARS runs). DEC + J was the best-fit model for *Triplaris/Ruprechtia* and *Platymiscium*, while for *Vachellia* and *Tachigali* DEC had the same likelihood as DEC + J (electronic supplementary material, table S9b-e). Because many Pseudomyrmecinae species are widespread, the inferred ancestral ranges are also wide (figure 2). The ancestral area of *Pseudomyrmex* includes Central and Northern South America (ML probability = 0.8), and the ancestral ranges of the *P. ferrugineus* and the *P. nigrocinctus* subgroups are Central America (ML prob. = 1 and = 0.95, respectively), matching the inferred ancestral range of their *Vachellia* host plants (ML prob. = 0.99). The *P. triplarinus* group originated in Northern South America (ML prob. = 0.8), matching the ancestral range of its host, *Triplaris* (ML prob. = 0.8), and the same holds for the *P. concolor* group (ML prob. = 0.9) and its host *Tachigali* (ML prob. = 0.75–1 depending on lineage). The *P. fortis* species group within the *P. sericeus* group evolved in Northern South America (ML prob. = 0.9), where some of its hosts also arose (*Triplaris* and some myrmecophytic lineages of *Platymiscium*). In *Pseudomyrmex*, the evolution of obligate plant nesting correlates with a reduction in the number of ancestral areas (figure 2), which is partially confirmed by range size analysis (electronic supplementary material, figure S11).

(d) Gains or losses of plant nesting in Pseudomyrmecinae and of domatia in their major plant hosts

Our ML and Bayesian reconstructions (electronic supplementary material, Material and Methods; and figures S5 and S6) of plant nesting in Pseudomyrmecinae strongly support 10 origins of obligate domatium living in this subfamily, including five in the genus *Pseudomyrmex* alone. This result was highly supported across methodological approaches (figure 2; electronic supplementary material, figures S5, S6 and S12; and also electronic supplementary material). Two independent origins of domatium living are supported within the *P. ferrugineus* group, one in the *P. ferrugineus* subgroup and one in the *P. nigrocinctus* subgroup (Bayesian prob. 0.98, ML prob. 0.97–0.99; electronic supplementary material, figures S5, S6 and S12). No loss of obligate plant nesting was detected. In the plants, we inferred single gains of domatia in *Vachellia* (electronic supplementary material, figures S7 and S12) and *Triplaris* (electronic supplementary material, figures S8 and S12), confirming previous results [14], and three gains of domatia in *Ruprechtia* (electronic supplementary material, figures S8 and S12), at least nine in *Tachigali* (electronic supplementary material, figures S9 and S12) and five in *Platymiscium* (electronic supplementary material, figure S10 and S12). No domatium loss was inferred. Stochastic trait mapping (electronic supplementary material, Materials and Methods; and electronic supplementary material, figure S12) confirmed the results obtained with other methods.

4. Discussion

(a) Macroevolutionary assembly of ant/plant symbioses

The expectation that highly age-discrepant partners would be rare turned out to be wrong, while our expectations that co-diversification would be seen only in relatively young

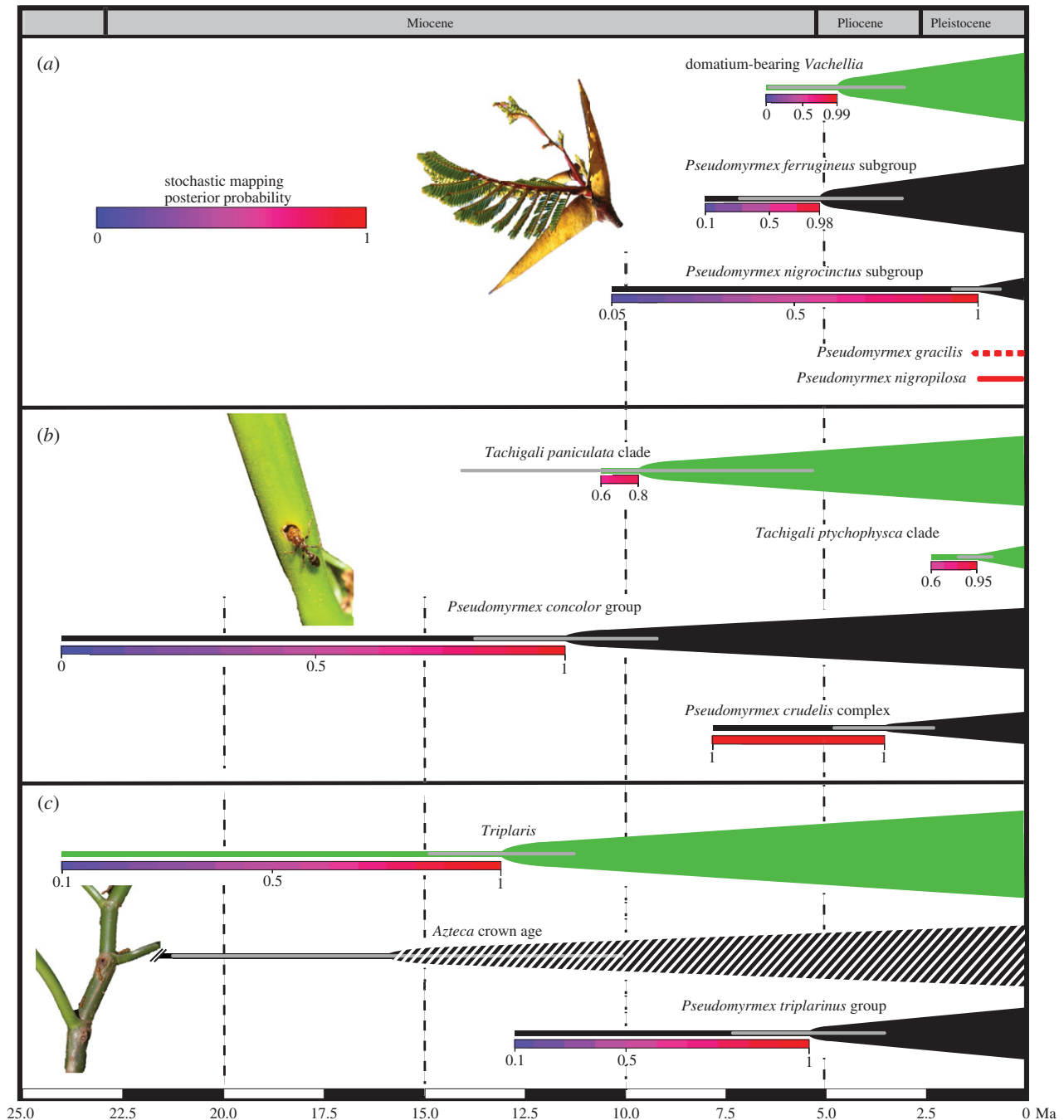


Figure 3. Macroevolutionary patterns of age and trait matching of interacting *Pseudomyrmex* ants and domatium-bearing plant lineages and hypothetical-associated evolutionary processes. (a) Co-radiation of *Vachellia* and the *P. ferrugineus* subgroup, followed by secondary colonization by mutualistic species of the *P. nigrocinctus* species complex, parasitic *P. nigropilosus* and the generalist *P. gracilis*. (b) Potential initial co-radiation of *Tachigali* and the *P. concolor* species group, followed by host broadening to other *Tachigali* lineages and secondary colonization of *Tachigali* by members of the *P. crudelis* species complex. (c) In domatium-bearing *Triplaris*, crown and stem ages and ancestral state reconstruction suggest that the ant mutualists (the *P. triplarinus* group) that currently nest in *Triplaris* domatia are younger by approximately 8 Myr than is domatium-presence in *Triplaris*, suggesting possible symbioses with other (earlier) ant species, such as *Azteca* whose crown age (banded) matches *Triplaris* and which sometimes forms symbioses with the latter (see S4). Grey error bars show the 95% CI from BEAST. Black (ants) or green (plant) bars depict stem branches. Colour gradient along the stem branch shows the posterior probability of a density plot summarizing 1000 stochastic simulations of trait evolution. See also the associated electronic supplementary material, figure S12. Below the arbitrary threshold of 0.5, the traits (domatium or domatium-nesting) are unlikely to have been present. (Online version in colour.)

clades and that parasitic species would be younger than mutualistic species were both met. Temporally matched radiation (co-radiation) of interacting clades has occurred in the *P. ferrugineus* group and its *Vachellia* host species in Central America (figure 3a). Most ant species in this group can nest in several *Vachellia* species, with the exception of *P. satanicus*, which seems restricted to *V. melanoceras* ([20,24]; figure 2). We found no obvious matching of the DNA tree topologies, suggesting the absence of co-speciation, and the branching

times of interacting species are not always temporally matched (figure 2), further pointing to host broadening and host switching. The limited dispersal ability of symbiotic ants and plants and their typically low specificity probably hamper co-speciation in ant/plant symbioses [12,14]. Reciprocally matching traits in *Vachellia* and their big-eyed ant symbionts include protein-rich food bodies (Beltian bodies) that are more effectively digested by *P. ferrugineus* ants than by generalist species [54], enlarged extrafloral nectaries

(EFNs) with post-secretory nectar sucrose hydrolysis and the ants' ability to feed on sucrose-poor nectar [55]. A novel finding of this study is that *P. nigrocinctus* and *P. particeps* form a distinct lineage much younger than the remaining *Vachellia* ants, which apparently colonized already domatium-possessing *Vachellia* species (figure 3a; electronic supplementary material, figures S5–S7). *Pseudomyrmex nigrocinctus* is widespread, occupying several *Vachellia* species, while *P. particeps* is known only from *V. allenii*, a species that can also be inhabited by *P. spinicola*, a member of the *P. ferrugineus* subgroup [20]. *Vachellia allenii* thus represents a clear case of symbiont broadening, with the younger ant species *P. particeps* now competing with *P. spinicola* for domatia to live in. Both *P. nigrocinctus* and *P. particeps* patrol their host plants aggressively and gather Beltian bodies and extrafloral nectar ([56]; P.S.W., personal observation), but the extent to which they have adapted nutritionally to *Vachellia*, perhaps with traits similar to those found in the *P. ferrugineus* subgroup [54,55], remains to be investigated.

Tachigali domatia, which evolved at least nine times (figure 2), arise from an enlargement of the leaf rachis (and in some cases also the inflorescence stem), which may be developmentally 'easy' and happen readily under selection pressure from domatium-nesting ants, in this case ants of the *P. concolor* group (figure 2), as long as founder queens can cover the distance between domatium-bearing and non-domatium-bearing species occurring sympatrically [57]. The repeated evolution of domatia in related *Tachigali* species provides a striking example of parallel evolution that results from recurrent colonization by *P. concolor* group. The significantly younger age of the *P. crudelis* species group (3.7 Ma versus 12.2 for the *P. concolor* group and 8 Ma for the main domatium-bearing *Tachigali* lineage) strongly suggests that it secondarily colonized *Tachigali* (figures 2, 3b; electronic supplementary material, figures S5, S6 and S9). Secondary colonization, such as that of *Vachellia* by the *P. nigrocinctus* species complex and of *Tachigali* by the *P. crudelis* species complex, results in symbiont broadening for the plants and enables entry into 'new adaptive zones' represented by the myrmecophytes. The *P. triplarinus* group is 5–8 Myr younger than its obligate host *Triplaris* (figures 2 and 3; electronic supplementary material, figures S5, S6, S8 and S12). Wide crown and stem age differences between the ant-housing genus *Triplaris* (Polygonaceae) and its obligate ant inhabitants, and stochastic trait mapping (figures 2 and 3; electronic supplementary material, figure S12), indicate that its domatium evolved earlier than the ants now occupying it, suggesting previous symbioses that dissolved. *Triplaris* might thus represent a later stage in the evolution of coevolution as envisioned by Ehrlich & Raven [58], namely the complete switching to a new partner. Partner replacement could come about through colonization of domatia by generalist plant-ants [59,60]. A potential candidate for an earlier symbiosis with *Triplaris* is *Azteca*, a clade whose crown age matches that of *Triplaris* (figure 3c, [61]) and which contains both *Triplaris* specialists [46,62] and infrequent occupants of *Triplaris* domatia [51]. Alternatively, the inferred domatium trait might be an exaptation that would only have been converted later into a domatium, or *Triplaris* might have been associated with (now extinct) stem lineage species of the *P. triplarinus* group.

(b) Recent colonization of mutualistic symbioses by parasitic ant species

Our time-calibrated phylogenetic framework for the evolution of big-eyed ants and their plant host groups reveals that

specialized mutualist species form well-defined clades, while parasite species consist of singletons (figure 2). Although the time of origin of a parasitic lifestyle in single species cannot be inferred, the relevant sister species divergence times imply that parasites evolved later than mutualists: *P. nigropilosus*, a specialist ant species that obligately nests in *Vachellia* and feeds on its food bodies and extrafloral nectar but does not protect it against herbivores or encroaching vegetation [27], split from its sister species *P. major* only 1.5 Ma. Similarly, *P. gracilis*, an arboreal generalist that occasionally occupies *Vachellia* and prevents queens of mutualistic ants from founding a new colony [63], split from the related species, *P. hospitalis*, only 1.7 Ma. Younger ages of ant parasites compared to mutualists are expected since mutualistic selection pressure must first have led to the evolution of domatia before parasitic ants could exploit these nesting structures. In all cases, we found that parasites evolved from generalists and not from mutualists, contrary to a common prediction in mutualism models [3], but consistent with previous phylogenetic analyses with less dense species sampling [47].

5. Conclusion

Our study reveals macroevolutionary patterns that may represent different stages in the evolution of ant/plant symbioses. Based on crown ages, we inferred co-diversification in the *Vachellia/Pseudomyrmex ferrugineus*-group system over a few million years and secondary and parallel colonizations of *Vachellia*, *Tachigali* and *Platymiscium* by other ant groups that entered new 'adaptive zones' (mutualistic or parasitic). In *Triplaris*, we present evidence that the current *Pseudomyrmex* partners are secondary colonists that displaced earlier symbiont species, possibly as a consequence of locally reduced abundances and competition among plant-ants for nesting sites. The repeated evolution of domatia in *Tachigali* (26 of its 54 species have domatia; figure 2) may provide an example of a guild, namely the *Pseudomyrmex concolor* species group, imposing selection pressures on related plant species. Altogether, our study reveals that assemblage of ant/plant symbioses has been highly dynamic, even in very specialized systems, such as the iconic Central American *Vachellia* and its thorn-domatium-nesting *P. ferrugineus* group.

Data accessibility. Data are available in TreeBase accessions S17550 (*Pseudomyrmecinae*) and Dryad accession <http://dx.doi.org/10.5061/dryad.5p51r> (plants).

Competing interests. We declare we have no competing interests.

Funding. This work was supported by a grant from the German Research Foundation (DFG), RE 603/20 and grants from the Society of Systematic Biologists and the American Association of Plant Taxonomy to G.C. Research on *Pseudomyrmecinae* ants was supported by a series of grants to P.S.W. from the US National Science Foundation, most recently DEB-1354996.

Acknowledgements. We thank Alex Wild, Heraldo Vasconcelos, Fabian Michelangeli and Ricardo Solar for photos, and curators of the herbaria of the Missouri Botanical Garden and the Catholic University of Quito for permission to isolate DNA from specimens under their care. We thank Rumsais Blatrix for a *Tachigali* leaf sample, Rosa Elena Andrade and Christian Feregrino for help in the lab, and Jeremy Aroles for proofreading the manuscript. P.S.W. extends gratitude to numerous collectors and curators who provided access to ant specimens, especially Gary Alpert, Woody Benson, Roberto Brandão, Stefan Cover, Fernando Fernández, Brian Fisher, John Lattke, Jack Longino, Bill MacKay, Roy Snelling and Jim Wetterer. We are grateful to the associate editor Megan Frederickson and three anonymous reviewers for critical comments that improved the manuscript.

References

- Bronstein JL, Alarcón R, Geber M. 2006 The evolution of plant–insect mutualisms. *New Phytol.* **172**, 412–428. (doi:10.1111/j.1469-8137.2006.01864.x)
- Frederickson ME. 2013 Rethinking mutualism stability: cheaters and the evolution of sanctions. *Q. Rev. Biol.* **88**, 269–295. (doi:10.1086/673757)
- Sachs JL, Simms EL. 2006 Pathways to mutualism breakdown. *Trends Ecol. Evol.* **21**, 585–592. (doi:10.1016/j.tree.2006.06.018)
- 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 Phytol.* **198**, 347–385. (doi:10.1111/nph.12150)
- Clark MA, Moran NA, Baumann P, Wernegreen JJ. 2000 Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. *Evolution* **54**, 517–525. (doi:10.1111/j.0014-3820.2000.tb00054.x)
- Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T. 2006 Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. *PLoS Biol.* **4**, e337. (doi:10.1371/journal.pbio.0040337)
- Jousselin E, Desdevises Y, Coeur d'Acier A. 2009 Fine-scale cospeciation between *Brachycaudus* and *Buchnera aphidicola*: bacterial genome helps define species and evolutionary relationships in aphids. *Proc. R. Soc. B* **276**, 187–196. (doi:10.1098/rspb.2008.0679)
- Cruaud A *et al.* 2012 An extreme case of plant–insect codiversification: figs and fig-pollinating wasps. *Syst. Biol.* **61**, 1029–1047. (doi:10.1093/sysbio/sys068)
- Bogler DJ, Neff JL, Simpson BB. 1995 Multiple origins of the yucca-yucca moth association. *Proc. Natl Acad. Sci. USA* **92**, 6864–6867. (doi:10.1073/pnas.92.15.6864)
- Smith CI, Pellmyr O, Althoff DM, Balcazar-Lara M, Leebens-Mack J, Seagraves KA. 2008 Pattern and timing of diversification in *Yucca* (Agavaceae): specialized pollination does not escalate rates of diversification. *Proc. R. Soc. B* **275**, 249–258. (doi:10.1098/rspb.2007.1405)
- Gu L, Luo Z, Zhang D, Renner SS. 2010 Pollination of *Rhodoleia championii* (Hamamelidaceae) in subtropical China. *Biotropica* **42**, 336–341. (doi:10.1111/j.1744-7429.2009.00585.x)
- Davidson DW, McKey D. 1993 The evolutionary ecology of symbiotic ant/plant relationships. *J. Hymenopt. Res.* **2**, 13–83.
- Frederickson ME, Greene MJ, Gordon DM. 2005 Ecology: 'Devil's gardens' bedevilled by ants. *Nature* **437**, 495–496. (doi:10.1038/437495a)
- Chomicki G, Renner SS. 2015 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. *New Phytol.* **207**, 411–424. (doi:10.1111/nph.13271)
- Chenuil A, McKey D. 1996 Molecular phylogenetic study of a myrmecophyte symbiosis: did *Leonardoxa*/ant associations diversify via cospeciation? *Mol. Phylogenet. Evol.* **6**, 270–286. (doi:10.1006/mpev.1996.0076)
- 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. *Mol. Ecol.* **13**, 1157–1171. (doi:10.1111/j.1365-294X.2004.02113.x)
- 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. (doi:10.1111/j.0014-3820.2004.tb01678.x)
- Gómez-Acevedo S, Rico-Arce L, Delgado-Salinas A, Magallón S, Eguarte LE. 2010 Neotropical mutualism between *Acacia* and *Pseudomyrmex*: phylogeny and divergence times. *Mol. Phylogenet. Evol.* **561**, 393–408. (doi:10.1016/j.jmpev.2010.03.018)
- Ward PS. 1991 Phylogenetic analysis of pseudomyrmecine ants associated with domatia-bearing plants. In *Ant-plant interactions* (eds CR Huxley, DF Cutler), pp. 335–352. New York, NY: Oxford University Press.
- Ward PS. 1993 Systematic studies on *Pseudomyrmex acacia*-ants Hymenoptera: Formicidae: Pseudomyrmecinae. *J. Hym. Res.* **2**, 117–168.
- Ward PS. 1999 Systematics, biogeography and host plant associations of the *Pseudomyrmex viduus* group Hymenoptera: Formicidae, *Triplaris*- and *Tachigali*-inhabiting ants. *Zool. J. Linn. Soc.* **126**, 451–540. (doi:10.1111/j.1096-3642.1999.tb00157.x)
- Ward PS, Downie DA. 2005 The ant subfamily Pseudomyrmecinae Hymenoptera: Formicidae: phylogeny and evolution of big-eyed arboreal ants. *Syst. Entomol.* **30**, 310–335. (doi:10.1111/j.1365-3113.2004.00281.x)
- AntCat. 2015 AntCat. An online catalog of the ants of the world. See <http://antcat.org> (accessed 24 April 2015).
- Janzen DH. 1974 *Swollen-thorn Acacias of Central America*. Washington, DC: Smithsonian Institution Press.
- Heil M, González-Teuber M, Clement LW, Kautz S, Verhaagh M, Silva Bueno JC. 2009 Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proc. Natl Acad. Sci. USA* **106**, 18 091–18 096. (doi:10.1073/pnas.0904304106)
- Hocking B. 1970 Insect associations with the swollen thorn acacias. *Trans. R. Entomol. Soc. Lond.* **122**, 211–255. (doi:10.1111/j.1365-2311.1970.tb00532.x)
- Janzen DH. 1975 *Pseudomyrmex nigropilosa*: a parasite of a mutualism. *Science* **188**, 936–937. (doi:10.1126/science.188.4191.936)
- Matzke NJ. 2012 Founder-event speciation in BioGeoBEARS package dramatically improves likelihoods and alters parameter inference in dispersal–extinction–cladogenesis DEC analyses. *Front. Biogeogr.* **4**, 210.
- Matzke NJ. 2014 Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Syst. Biol.* **63**, 951–970. (doi:10.1093/sysbio/syu056)
- Sanchez A, Kron KA. 2008 Phylogenetics of Polygonaceae with an emphasis on the evolution of Eriogonoideae. *Syst. Bot.* **33**, 87–96. (doi:10.1600/036364408783887456)
- Saslis-Lagoudakis C, Chase MW, Robinson DN, Russell SJ, Klitgaard BB. 2008 Phylogenetics of neotropical *Platymiscium* Leguminosae: Dalbergieae: systematics, divergence times, and biogeography inferred from nuclear ribosomal and plastid DNA sequence data. *Am. J. Bot.* **95**, 1270–1286. (doi:10.3732/ajb.0800101)
- Chomicki G, Renner SS. 2015 Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museumics. *New Phytol.* **205**, 526–532. (doi:10.1111/nph.13163)
- Katoh K, Standley DM. 2013 MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780. (doi:10.1093/molbev/mst010)
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997 The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* **24**, 4876–4882. (doi:10.1093/nar/25.24.4876)
- Maddison WP, Maddison DR. 2011 Mesquite 2.75: a modular system for evolutionary analysis. See <http://mesquiteproject.org> (accessed 1 April 2015).
- Maddison DR, Maddison WP. 2005 *MacClade 4: analysis of phylogeny and character evolution*. Version 4.08. Sunderland, MA: Sinauer Press.
- Stamatakis A. 2014 RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **309**, 1312–1313. (doi:10.1093/bioinformatics/bt0033)
- Bazinnet AL, Zwickl DJ, Cummings MP. 2014 A gateway for phylogenetic analysis powered by grid computing featuring GARLI 2.0. *Syst. Biol.* **63**, 812–818. (doi:10.1093/sysbio/syu031)
- Lanfear R, Calcott B, Ho SY, Guindon S. 2012 PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* **296**, 1695–1701. (doi:10.1093/molbev/mss020)
- Ronquist F *et al.* 2012 MrBayes 3.2: efficient Bayesian phylogenetic inference and model

- choice across a large model space. *Syst. Biol.* **613**, 539–542. (doi:10.1093/sysbio/sys029)
41. Darriba D, Taboada GL, Doallo R, Posada D. 2012 jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* **9**, 772. (doi:10.1038/nmeth.2109)
 42. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014 BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comp. Biol.* **104**, e1003537. (doi:10.1371/journal.pcbi.1003537)
 43. Rambaut A, Drummond AJ. 2007 Tracer—MCMC trace analysis tool version v1.5. See <http://beast.bio.ed.ac.uk>.
 44. Janzen DH. 1966 Coevolution of mutualism between ants and acacias in Central America. *Evolution* **20**, 249–275. (doi:10.2307/2406628)
 45. Wheeler WM, Bailey IW. 1920 The feeding habits of pseudomyrmine and other ants. *Trans. Am. Philos. Soc.* **22**, 235–279. (doi:10.2307/1005485)
 46. Wheeler WM. 1942 Studies of Neotropical ant-plants and their ants. *Bull. Mus. Comp. Zool.* **90**, 1–262.
 47. Kautz S, Lumbsch HT, Ward PS, Heil M. 2009 How to prevent cheating: a digestive specialization ties mutualistic plant-ants to their ant-plant partners. *Evolution* **63**, 839–853. (doi:10.1111/j.1558-5646.2008.00594.x)
 48. Paradis E, Claude J, Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290. (doi:10.1093/bioinformatics/btg412)
 49. Pagel M, Meade A. 2013 *BayesTraits v. 2.0*. Reading, MA: University of Reading. See <http://www.evolution.rdg.ac.uk/BayesTraits.html>.
 50. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005 Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978. (doi:10.1002/joc.1276)
 51. Sanchez A. 2011 Evolutionary relationships in Polygonaceae with an emphasis on *Triplaris*. PhD thesis, Wake Forest University, NC, USA
 52. Moreau CS, Bell CD, Villa R, Archibald SB, Pierce N. 2006 Phylogeny of ants: diversification in the age of angiosperms. *Science* **312**, 101–104. (doi:10.1126/science.1124891)
 53. 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. (doi:10.1111/evo.12105)
 54. Orona-Tamayo D, Wielsch N, Blanco-Labra A, Svatos A, Fariás-Rodríguez R, Heil M. 2013 Exclusive rewards in mutualisms: ant proteases and plant protease inhibitors create a lock–key system to protect *Acacia* food bodies from exploitation. *Mol. Ecol.* **2215**, 4087–4100. (doi:10.1111/mec.12320)
 55. Heil M, Rattke J, Boland W. 2005 Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* **308**, 560–563. (doi:10.1126/science.1107536)
 56. Beulig ML, Janzen DH. 1969 Variation in behavior among obligate acacia-ants from the same colony (*Pseudomyrmex nigrocincta*). *J. Kansas Entomol. Soc.* **42**, 58–67.
 57. van der Werff H. 2008 A synopsis of the genus *Tachigali* (Leguminosae: Caesalpinioideae) in Northern South America. *Ann. Miss. Bot Gard.* **95**, 618–661. (doi:10.3417/2007159)
 58. Ehrlich PR, Raven PH. 1964 Butterflies and plants: a study in coevolution. *Evolution* **18**, 586–608. (doi:10.2307/2406212)
 59. McKey D. 1984 Interaction of the ant-plant *Leonardoxa africana* Caesalpinaceae with its obligate inhabitants in a rainforest in Cameroon. *Biotropica* **16**, 81–99. (doi:10.2307/2387840)
 60. Maschwitz U, Fiala B, Davies SJ, Linsenmair KE. 1996 A South-East Asian myrmecophyte with two alternative inhabitants: *Camponotus* or *Crematogaster* as partners of *Macaranga lamellata*. *Ecotropica* **2**, 29–40.
 61. Pringle EG, Ramirez SR, Bonebrake TC, Gordon DM, Dirzo R. 2012 Diversification and phylogeographic structure in widespread *Azteca* plant-ants from the northern Neotropics. *Mol. Ecol.* **21**, 3576–3592. (doi:10.1111/j.1365-294X.2012.05618.x)
 62. Longino JT. 2007 A taxonomic review of the genus *Azteca* (Hymenoptera: Formicidae) in Costa Rica and a global revision of the aurita group. *Zootaxa* **1491**, 1–63.
 63. Clement LW, Köppen S, Brand WA, Heil M. 2008 Strategies of a parasite of the ant–*Acacia* mutualism. *Behav. Ecol. Sociobiol.* **26**, 953–962. (doi:10.1007/s00265-007-0520-1)