Ant–plant interactions evolved through increasing interdependence

Matthew P. Nelsen[a,1], Richard H. Ree[a], and Corrie S. Moreau[a]

[a]Department of Science and Education, Integrative Research Center, Field Museum of Natural History, Chicago, IL 60605

Edited by May R. Berenbaum, University of Illinois at Urbana–Champaign, Urbana, IL, and approved October 22, 2018 (received for review November 13, 2017)

Ant–plant interactions are diverse and abundant and include classic models in the study of mutualism and other biotic interactions. By estimating a time-scaled phylogeny of more than 1,700 ant species and a time-scaled phylogeny of more than 10,000 plant genera, we infer when and how interactions between ants and plants evolved and assess their macroevolutionary consequences. We estimate that ant–plant interactions originated in the Mesozoic, when predatory, ground-inhabiting ants first began foraging arboreally. This served as an evolutionary precursor to the use of plant-derived food sources, a dietary transition that likely preceded the evolution of extrafloral nectaries and elaiosomes. Transitions to a strict, plant-derived diet occurred in the Cenozoic, and optimal models of shifts between strict predation and herbivory include omnivory as an intermediate step. Arboreal nesting largely evolved from arboreally foraging lineages relying on a partially or entirely plant-based diet, and was initiated in the Mesozoic, preceding the evolution of domatia. Previous work has suggested enhanced diversification in plants with specialized ant-associated traits, but it appears that for ants, living and feeding on plants does not affect ant diversification. Together, the evidence suggests that ants and plants increasingly relied on one another and incrementally evolved more intricate associations with different macroevolutionary consequences as angiosperms increased their ecological dominance.

mutualism | coevolution | angiosperms | macroevolution

Biotic interactions are ubiquitous, yet their evolutionary origins and macroevolutionary consequences are often poorly understood. Placing the evolutionary history of interacting lineages and their associated traits in a temporal framework can help reveal the reciprocal dynamics of coevolution by identifying the sequence and tempo of events in the evolution of interactions, and the influence of these events on lineage diversification. The diversification of numerous terrestrial lineages is attributed, at least in part, to the decline of the Mesozoic, gymnosperm-dominated vegetation and the rise of the Cenozoic, angiosperm-dominated flora (refs. 1 and 2 and therein). Rapid diversification is commonly associated with the exploitation of novel niches and resources (3, 4). Unique aspects of angiosperm reproductive and vegetative biology, coupled with increased ecosystem complexity, provided numerous novel niches and resources that interacting lineages could exploit, whereas the evolution of angiosperms with high transpiration capacities altered the climate and enabled the development and expansion of tropical rainforests (2).

Ants (Hymenoptera: Formicidae) are among the most abundant insects on Earth (5). Diversification of the major ant lineages increased during the global transition to angiosperm dominance (1, 6, 7), when ancestrally carnivorous ant lineages that occupied the soil or litter layer are thought to have incorporated plant-based food sources (PBFS) into their diet (8), setting the stage for their expansion into arboreal habitats and previously unexploited ecosystems (9). The increased ecological opportunity provided may therefore have enhanced diversification of lineages able to exploit these resources. In addition, acquisition of plant-derived food sources may be more metabolically efficient, as these sedentary food sources do not require energy to be expended on subduing prey (10). As elevated diversification rates have been inferred in various herbivorous and arboreal clades of animals and epiphytic plants (11–17), we thus predict that ant lineages that rely on plants for food and habitat may also show evidence of faster diversification (cf. ref. 18).

Ants interact with a range of tissues and structures across vascular plants, with the strength of these interactions varying from diffuse to highly specific (19–23). The three most common structures are elaiosomes, extrafloral nectaries (EFNs), and domatia. Elaiosomes are lipid-rich seed appendages found in more than 11,000 species across 77 families of angiosperms, which ants use as a food source while protecting and dispersing the seeds to nutrient-rich locations and reducing parent–offspring competition (22–24). EFNs (including foliar nectaries in ferns) occur in nearly 4,000 species in more than 100 families of angiosperms and ferns, providing nutrition to ants, whereas the ants protect the host plant from herbivores and pathogens (19, 21). Domatia are known from nearly 700 vascular plant species in 50 families and are specialized structures in which ants live and defend the host plant (20, 25). Ants may also obtain food from plants indirectly, by farming hemipterans for their liquid exudates (honeydew), often within these domatia (26–28). The ecological advantages provided to plants through their interactions with ants may therefore be expected to confer success over macroevolutionary timescales. Previous work has yielded substantial insight, suggesting that EFN and elaiosome-forming plant lineages are associated with enhanced diversification rates (22, 29), whereas domatia formation is not expected to

Significance

Flowering plants are thought to have facilitated ant diversification by providing novel sources of food and habitat, and ants to have facilitated plant diversification by providing defense and dispersal. We test these hypotheses by inferring when and how interactions between ants and plants evolved, and examine their macroevolutionary consequences in ants. Although plants with ant-associated traits have been shown to have higher diversification rates, here we demonstrate that living and feeding on plants does not affect ant diversification. Ants used plants as food and nesting sources substantially before the evolution of ant-associated structures. Arboreal foraging preceded use of plant-derived food sources. Arboreal nesting then largely evolved from arboreally foraging ancestors. Finally, omnivory served as an evolutionary link between predation and herbivory.

Author contributions: M.P.N., R.H.R., and C.S.M. designed research; M.P.N. performed research; M.P.N. analyzed data; and M.P.N., R.H.R., and C.S.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: mpnelsen@gmail.com.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1719794115/-/DCSupplemental.

influence diversification rate (20). The fossil record of ants and plants is abundant and diverse, but their interactions are rarely preserved; as a consequence, comparative analyses of time-calibrated phylogenies may clarify their origins and interactions. Fossils reveal that EFNs and ant–hemipteran associations occurred as early as the Eocene (30, 31). Molecular clock analyses suggest that plant clades forming EFNs evolved by the Eocene (32–34), Late Cretaceous origins of elaiosome-bearing families (23, 35), and Neogene origins of domatia (20). However, the coarse phylogenetic granularity (family-level) or the restriction to specific traits or clades leaves open many questions about the age and evolutionary pathways through which ant–plant interactions evolved.

Here we investigate the evolution of these interactions at a broad phylogenetic scale with a variety of analyses that complement earlier work. Specifically, we ask whether increased diversification in ants is associated with use of plants as food sources or habitat. We also investigate when plants evolved the potential to form ant-associated structures, both in geological time and in relation to the origin of plant use by ants.

Results

Temporal Evolution of Ant-Associated Traits in Plants. We inferred a time-scaled phylogeny of 10,785 vascular plant genera using alignments from ref. 36. Genera with species forming EFNs, elaiosomes, and domatia (20, 21, 23) were broadly distributed (Fig. 1). Analyses of phylogenetic signal (37) suggested that the measured signal of EFNs (D = 0.584), elaiosomes (D = 0.400), and domatia (D = 0.736) was significantly different ($P < 0.001$) from random (D = 1) and Brownian motion (D = 0). Our maximum likelihood (ML) ancestral state reconstruction (ASR) suggested the potential to form EFNs initially evolved in the mid-Cretaceous, with most acquisitions of these traits occurring through the Paleogene and Neogene (Fig. 2). The potential to form elaiosomes is inferred to have first evolved near the Cretaceous–Paleogene boundary, with most transitions occurring through the Paleogene and Neogene (Fig. 2). In contrast, the potential to form domatia appears to have lagged behind, with initial evolution occurring in the latest Paleogene and most gains occurring in the Neogene (Fig. 2). Finally, the evolution of the potential to form EFNs and domatia in angiosperms is inferred to have preceded their evolution in ferns (*SI Appendix*, Figs. S1–S3).

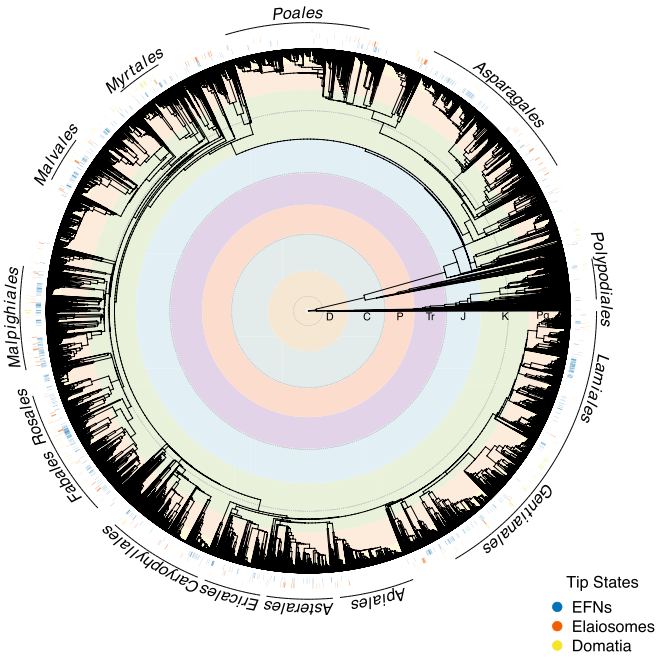

Fig. 1. The phylogenetic distribution of ant-associated traits across 10,785 vascular plant genera. Shaded bands behind the phylogeny correspond to geological periods, whereas dashed lines occur at 100-Ma time intervals. The presence (color) or total absence (blank) of species possessing these traits is indicated in rings surrounding the phylogeny. Higher-level taxonomy is plotted for larger clades.

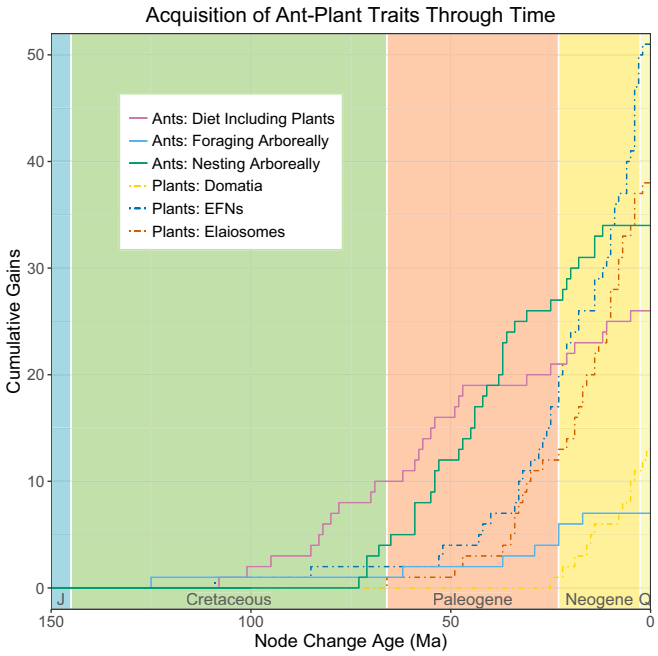

Fig. 2. The evolution of plant-associated traits in ants (solid lines) and the potential to form ant-associated traits in plants (dashed lines) through time. Terminal taxa that possessed a change between the tip and their parent node were not included to facilitate the visualization of earlier transitions. Ant lineages that at least partially fed on plant material or lived arboreally were coded as using plants as a food source and living arboreally. Vertical shading corresponds to geological period.

Evolution of Plant-Dependence in Ants. We inferred a time-scaled, species-level phylogeny of 1,730 ant species. The Bayesian analysis of macroevolutionary mixtures revealed substantial heterogeneity in speciation rates across the phylogeny (Fig. 3). Associations with plants were indicated by coding taxa for diet, foraging, and nesting habitats, based on genus-level designations in a previously compiled dataset (18). No evidence for a significant association among diet, foraging, or nesting location and speciation, extinction, or net diversification rate was recovered by phylogenetic ANOVA (38), hidden state speciation and extinction, or structured rate permutations on phylogenies (39) analyses (*SI Appendix*, Tables S1–S3). These trends were consistent regardless of character coding scheme used. Analyses of phylogenetic change in diet, foraging, and habitat location independent of diversification supported a model in which the evolution of all three traits were correlated (*SI Appendix*, Table S4). Here, we prefer to emphasize the sequence of events and transition rates derived from this model, rather than the implied correlated evolution of these traits. Ancestral state reconstruction under this model suggested ants began foraging arboreally and incorporating PBFS into their diets during the Early Cretaceous, whereas arboreal nesting originated near the end of the Late Cretaceous [Figs. 2 and 3 (lineages within Myrmicinae, Formicinae and Dolichoderinae, for instance)]. However, most transitions in diet and nesting location were

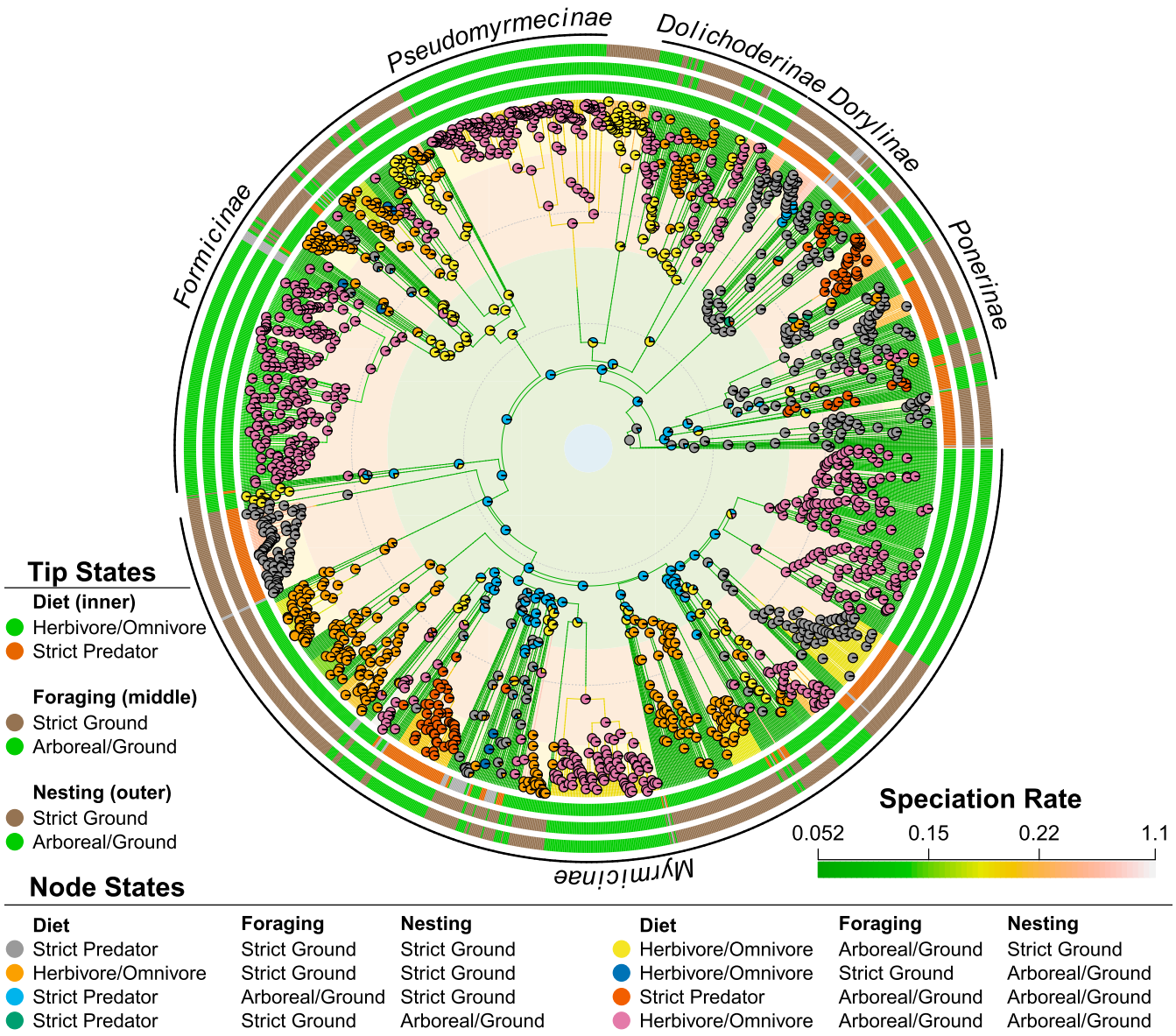

Fig. 3. Time-scaled ant phylogeny inferred from 1,746 species (1,731 Formicidae), with representatives from 85% of extant ant genera. Branches are shaded by Bayesian analysis of macroevolutionary mixtures-estimated speciation rate. Shaded bands underneath the phylogeny correspond to geological periods, with dashed lines at 50-Ma intervals. Pie charts overlaying nodes indicate the proportional probability of an individual state. Diet, foraging, and nesting location is provided in rings surrounding the tips, with the absence of information on a trait indicated in gray.

confined to the Cenozoic. Arboreal foraging evolved early in the ants, but transitions were relatively uncommon and occurred most frequently in predatory lineages (Fig. 3 and *SI Appendix*, Table S5). These predatory, arboreally foraging lineages were the earliest and most frequent to evolve any sort of reliance on PBFS. The earliest transitions to the dietary inclusion of PBFS appear to be derived from a single transition to arboreal foraging; as a consequence, this pattern (pseudoreplicated burst) of evolution makes it difficult to disentangle whether the evolution of these traits is functionally correlated or coincidental (40). Once diets included PBFS, reversions to a strict predatory diet were very rare (Fig. 3 and *SI Appendix*, Table S5). Finally, transitions to arboreal nesting were primarily derived from multiple arboreally foraging lineages that incorporated PBFS into their diet [Fig. 3 (lineages within Myrmicinae, Formicinae and Dolichoderinae, for instance); *SI Appendix*, Table S5].

We then assessed whether omnivory was a prerequisite for the evolution of strict plant-based diet by using maximum likelihood to estimate transition rates and ancestral states under different models of evolution. The model prohibiting direct transitions between predatory and strict plant-based diets was suggested as the best fit [ΔAICc (Akaike Information Criterion corrected for small sample sizes) > 1.95; *SI Appendix*, Table S6]. Three-state diet reconstructions suggested a predatory most recent common ancestor of Formicidae, with an initial shift to omnivory suggested in the Early Cretaceous, and the earliest transition to a strict plant-derived diet occurred in the Late Cretaceous (*SI Appendix*, Figs. S4–S8). Subsequent shifts to a strict plant-derived diet were primarily confined to the past 75 Ma.

Discussion

Evolutionary Use of PBFS by Ants. We infer that arboreal foraging in ants preceded the transition from a strict predatory habit to a diet including plant-derived food sources, implying that these

food sources were collected from the plant itself (including arboreal tending of sap-sucking insects), rather than from fallen plant tissue. Ant use of plants for food likely preceded the evolution of specialized food structures (EFNs and elaiosomes) in plants. These "early adopters" of the arboreal habitat may have first begun feeding on sap from cuts and wounds in the vegetation (41). In addition, many ants use hemipterans both for their honeydew as well as for prey (42); trophobiotic interactions between ants and aphids are thought to have originated as predator–prey relationships, which transitioned to mutualisms when aphids offered honeydew as a food source (43), and the same may apply to other ant–hemipteran interactions. Sap-sucking hemipterans evolved during or before the end of the Triassic (ref. 44 and therein), substantially predating ants, and the phylogenetic position of many trophobiotic clades (42, 45) is loosely consistent with early omnivore ant lineages relying partially on honeydew. It thus seems reasonable to suspect that early ants first transitioned to omnivory by opportunistically using sap from plant wounds and/or hemipteran honeydew before shifting fully to herbivory, as plants evolved specialized food sources for ants in return for defense and dispersal.

Evolution of Obligate Herbivory. It remains unclear why it took more than 50 Ma for ants to specialize entirely on plant-derived food sources. Alternate dietary codings consistently revealed a relatively late transition to a strictly herbivorous diet (additional files in the Dryad repository). Our analyses suggest that several ant clades evolved a strict reliance on plant-associated food sources from omnivorous ancestors. The first to do so was a myrmicine subclade of fungus-farming ants (*SI Appendix*, Figs. S4–S8), whose adults feed on leaf sap and adults and juveniles on fungi (ref. 46 and therein); the transition was thus not facilitated by EFNs or elaiosomes. Several physiological challenges are encountered when foraging in exposed locations and consuming a plant-based diet, which may have imposed significant evolutionary constraints. In addition, food sources such as sap from plant wounds and hemipteran honeydew may have not been limiting until consumers achieved high abundance; the subsequent evolution of specialized food sources, such as EFNs and elaiosomes, may have then enabled certain omnivorous lineages to evolve a strict reliance on plant-derived food sources.

Evolution of Arboreal Nesting. Our reconstructions show an evolutionary sequence in which a strictly predatory, ground-inhabiting ant lineage first transitioned to arboreal foraging, then incorporated plant-based foods into their diet, and finally began nesting arboreally. In plants, we infer that domatia evolved rather long after the origins of arboreal nesting, near the start of the Cenozoic (Fig. 2; see also ref. 20). Several large, monophyletic clades of strict, arboreal nesting ants predate the evolution of domatia; thus, we anticipate this finding will remain robust to a refined, species-level coding. The reasons for this temporal mismatch are unclear. Although some have suggested that ants have nested in domatia since the Cretaceous (47), no fossil data confirm this supposition. Numerous extant, arboreally nesting ant species use unspecialized, preformed openings in trees or beneath epiphytic plants and their substrate, or construct their own nesting structures, such as carton or silk nests (48, 49). Thus, numerous options likely existed for arboreally nesting ants before the evolution of domatia, with recent work demonstrating that domatia nesting in acacia ants evolved from ancestors nesting in dead twigs (50). Domatia are thought to have evolved through a number of distinct pathways involving a range of plant organs, morphological traits, and even the presence of hemipterans, which are thought to have served as domatial precursors or predisposed plant lineages to their formation (20, 51, 52).

The Age of Ant-Associated Traits in Plants. The phylogenetic resolution (genera) of our dataset limits the precision and certainty with which we can infer the origins of plant traits. Therefore, our binary scoring of the trait (EFNs, elaiosomes, or domatia) being present or not in at least one species of a genus means that our ASRs are best interpreted as an analysis of the potential for the trait to exist. Thus, the potential to form EFNs may have evolved as early as the mid-Cretaceous; however, it seems likely that their actual origin was much later. Chiefly, a rich record of vegetative plant fossils provides no evidence for the occurrence of EFNs before the late Eocene (30). Previous ancestral state estimates at the species level have shown that domatia and EFNs frequently evolved much later, typically within individual plant genera, and rarely are ancestral across larger, older clades (20, 29, 32). These analyses also showed that the presence of EFNs within a densely sampled clade is often marked by homoplasy. If the rate of gain and/or loss of EFNs has been consistently high in clades capable of producing them, they might have occurred ancestrally, but ephemerally. Our reconstruction of the evolutionary potential for these traits, although naive, nevertheless raises the possibility that EFNs and elaiosomes may have deeper ancestry than is commonly thought and motivates closer scrutiny of their evolution. Finally, the nonrandom distribution of these traits (here and refs. 21 and 29) suggests the presence of a genomic predisposition in these lineages enabling trait evolution, the presence of common selective agent precipitating their evolution, or a combination thereof.

Diversification of Ants Using PBFS and Arboreal Habitats. Contrary to observations in many other organismal groups, use of plants for food or habitat does not confer enhanced diversification rates in ants. A number of factors may be responsible for these discrepancies. First, PBFS typically used by ants (nectar, honeydew) are low in nutritional value; as a consequence, these lineages may be competitively inferior or devote fewer resources to reproduction or dispersal. Occupation of arboreal habitats exposes ants to both increased physiological challenges (desiccation) and predation; as a consequence, these may have neutral or negative effects on fitness and not enhance diversification. More generally, diversification rates may have been elevated during initial exploitation of novel plant-based resources, but as resources were diminished and niches filled, increased competition may have hampered diversification, whereas priority effects linked to plant-based resources may produce nonuniform clade-level responses to independent invasions of a niche (53).

Why Do Diversification Rate Patterns Differ Between Ants and Plants? Although theory and evidence suggest that biotic interactions are capable of influencing the dynamics of diversification, our results indicate that hypothesized beneficial effects on the fitness of interacting organisms may not be conferred to all interacting lineages at macroevolutionary scales (53). Benefits provided to plants by ants at the organismal level appear to translate to a macroevolutionary currency in which these plant lineages exhibit enhanced diversification rates (23, 29, 32). It is also worth considering the differences in how traits, our chosen indicators for ant–plant interactions, were scored for each group. For ants, plant-based food represents an amalgamation of food items that includes EFNs and elaiosomes, but also food bodies and hemipteran honeydew, among others. As a consequence, comparisons were not equivalent; for example, we were unable to strictly juxtapose diversification rates of elaiosome-feeding ants with elaiosome-producing plants. The ant coding scheme also included all ant taxa using plants; thus, mutualists and nonmutualists were included. In addition, phylogenetic scale can be important in detecting these processes. Plants may have facilitated diversification in specific lineages or windows of time, but

they do not universally explain ant diversification, highlighting the ecological and evolutionary complexity of ant diversification.

The Evolution of Ant–Plant Interactions. Here we suggest that although ant–plant interactions are characterized by temporal discordance, the evolution of these associations is likely tightly linked and evolved sequentially, but with asymmetric macroevolutionary consequences. Ants nested in plants and fed on plant-derived resources before the evolution of specialized plant structures such as EFNs, elaiosomes, and domatia, as would be expected. Nesting in plants before the evolution of domatia likely occurred in preexisting holes and gaps in plants or under epiphytes. The earliest omnivorous ants likely foraged arboreally and used plant sap and/or hemipteran honeydew as food sources. Ants relying strictly on a plant-based diet were derived from omnivorous lineages, with the earliest transition occurring more than 50 Ma after the origin of ants. Finally, although previous work suggests that plant lineages producing some of these ant-associated structures exhibited elevated diversification rates (23, 29, 32), our work demonstrates that ant lineages relying on plants for food, foraging, and nesting are not associated with enhanced diversification rates. This further suggests that biotic factors are capable of acting as agents of macroevolutionary change, but that the macroevolutionary consequences of these interactions cannot be universally predicted by effects at the organismic level (53). Furthermore, although ant–plant interactions originated in the Mesozoic, the evolution of specialized ant-associated structures and a strictly plant-derived diet likely evolved during the Cenozoic. Thus, as angiosperms achieved ecological dominance, ants and plants evolved more intricate associations with an increased interdependence on one another.

Methods

Phylogeny: Formicidae. NCBI was queried for Formicidae accessions derived from 12 loci, and a stepwise alignment procedure guided by higher-level taxonomy was used to construct a global alignment. Alignments from individual loci were concatenated into a 9,714-bp alignment, and PartitionFinder (54) was employed to identify the optimal partitioning strategy as determined by the Bayesian Information Criterion (BIC). A partitioned ML analysis using the general time-reversible categorical (GTRCAT) approximation was performed using randomized axelerated maximum likelihood (RAxML) on the Cyberinfrastructure for Phylogenetic Research (CIPRES) cluster (55). Minimum age constraints were derived from a previous synthesis (56). Fifty-one fossils were included as minimum age constraints (*SI Appendix*, Table S7), and the root node, representing the crown node of Aculeata, was fixed to 185 Ma ago, as implemented in previous ant phylogenetic studies (57). The penalized likelihood algorithm, as implemented in treePL (58), was used to timescale the tree.

ASR and Diversification Rate Analyses: Formicidae. Species richness estimates for extant genera were retrieved from AntWiki (www.antwiki.org/wiki/Species_Accounts), and subsequently used with our time-scaled phylogeny to estimate diversification rates across Formicidae in Bayesian analysis of macroevolutionary mixtures (59). Genus-specific sampling fractions were incorporated to account for incomplete sampling. Genus-level diet, foraging, and nesting locations were derived from a previous compilation (18), and character states applied to all species in a genus or genus-level group. Trait states in the original database were assigned to each genus based on literature records, and genera considered polymorphic if at least 10% of the examined species were in a conflicting state (18). Of the 1,731 ant taxa included, relatively few contained no information (diet, 67; foraging, 18; nesting, 20), whereas a larger number were polymorphic or ambiguous (diet, 245; foraging, 694; nesting, 589). Several coding schemes were created to accommodate taxa with questionable, missing, or multiple character states. In addition, dietary coding schemes with three states were also analyzed with a constrained transition matrix, which prevented direct transitions between herbivory and predation, forcing transitions to pass first through omnivory ("no02direct"). Marginal ancestral state estimates were then reconstructed using the "all rates different" (ARD) model in package corHMM (60) with the rayDISC function. We then also tested for the correlated evolution among diet, foraging, and nesting datasets, using the correlated path model (61) (a generalization of ref. 62) in corHMM (60) under the ARD model of evolution. We inferred when acquisitions of plant-associated traits occurred by dividing the tree into 1-Ma increments, and summing nonterminal nodes in which the most-likely state possessed the trait of interest and was descended from a node whose most-likely state lacked the trait of interest. As our interest was in documenting gains, rather than losses, this represents a cumulative total of gains of the trait through time. Summaries were made from the most-likely states derived from the best-fitting correlated paths model. We then tested for associations between character states and diversification rates in a number of ways, including a genus-level phylogenetic ANOVA (38), hidden state speciation and extinction analyses (63), and structured rate permutations on phylogenies analyses (39).

Phylogeny: Plants. Streptophyte genus-level alignments (36) were used to infer a phylogeny of vascular plants. Higher-level taxonomy was used as a topological constraint, and a partitioned ML analysis run in ExaML (64), constraining the monophyly of clades at or above the rank of family. The resulting tree consisted of 10,785 genera, which was then made ultrametric in treePL, using the mean ages of shared nodes in the phylogeny of (65) as constraints.

ASR Analyses: Plants. To identify plant genera that ants use as food sources (EFNs, elaiosomes) or form domatia, we used preexisting species or genus lists (20, 23, 66). These databases documented the presence of traits; as a consequence, taxa not included were scored as lacking the trait. Our phylogeny was linked to 84% (711/846), 86% (286/334), 90% (143/159) of genera known to possess EFNs, elaiosomes, and domatia, respectively. Any genus containing a species possessing these was coded as forming these (29), whereas genera not linked to trait databases were coded as lacking the trait. We measured phylogenetic signal [D-statistic (37)] in caper (67) and assessed significance through 1,000 permutations. ASR analyses were then conducted as earlier, using the ARD model.

Associated files are included in the Dryad repository and GitHub, and additional details provided in the *SI Appendix*.

ACKNOWLEDGMENTS. We thank Benjamin Blanchard and Shauna Price for many helpful conversations and for reading a previous version to greatly improve this study. We also express our gratitude to the reviewers and handling editor for their constructive feedback, which greatly improved the manuscript. M.P.N. thanks the Negaunee Foundation Interdisciplinary Postdoctoral Fellowship at the Field Museum of Natural History for support. R.H.R. and C.S.M. thank the National Science Foundation for research support Awards (NSF EF-1208727 to R.H.R.; NSF DEB 1442316 and NSF IOS 1354193 to C.S.M.).

1. 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.
2. Boyce CK, Lee J-E, Feild TS, Brodribb TJ, Zwieniecki MA (2010) Angiosperms helped put the rain in the rainforests: The impact of plant physiological evolution on tropical biodiversity. *Ann Mo Bot Gard* 97:527–540.
3. Simpson GG (1944) *Tempo and Mode in Evolution* (Columbia Univ Press, New York).
4. Schluter D (2000) *The Ecology of Adaptive Radiation* (Oxford Univ Press, Oxford).
5. Wilson EO (1988) The diversity of life. *Earth '88*, ed de Blij HJ (National Geographic Society, Washington, DC), pp 68–81.
6. Moreau CS, Bell CD (2011) Fossil cross-validation of the dated ant phylogeny (Hymenoptera: Formicidae). *Entomol Am* 117:127–133.
7. 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.
8. Lucky A, Trautwein MD, Guénard BS, Weiser MD, Dunn RR (2013) Tracing the rise of ants: Out of the ground. *PLoS One* 8:e84012.
9. Wilson EO, Hölldobler B (2005) The rise of the ants: A phylogenetic and ecological explanation. *Proc Natl Acad Sci USA* 102:7411–7414.
10. Sih A, Christensen B (2001) Optimal diet theory: When does it work, and when and why does it fail? *Anim Behav* 61:379–390.
11. Schuettpelz E, Pryer KM (2009) Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. *Proc Natl Acad Sci USA* 106:11200–11205.
12. Mitter C, Farrell B, Wiegmann B (1988) The phylogenetic study of adaptive zones: Has phytophagy promoted insect diversification? *Am Nat* 132:107–128.
13. Price SA, Hopkins SSB, Smith KK, Roth VL (2012) Tempo of trophic evolution and its impact on mammalian diversification. *Proc Natl Acad Sci USA* 109:7008–7012.
14. Wiens JJ, Lapoint RT, Whiteman NK (2015) Herbivory increases diversification across insect clades. *Nat Commun* 6:8370.
15. Burin G, Kissling WD, Guimarães PR, Jr, Şekercioğlu ÇH, Quental TB (2016) Omnivory in birds is a macroevolutionary sink. *Nat Commun* 7:11250.
16. Moen DS, Wiens JJ (2017) Microhabitat and climatic niche change explain patterns of diversification among frog families. *Am Nat* 190:29–44.
17. Poore AGB, Ahyong ST, Lowry JK, Sotka EE (2017) Plant feeding promotes diversification in the Crustacea. *Proc Natl Acad Sci USA* 114:8829–8834.
18. Blanchard BD, Moreau CS (2017) Defensive traits exhibit an evolutionary trade-off and drive diversification in ants. *Evolution* 71:315–328.

19. Bronstein JL, Alarcón R, Geber M (2006) The evolution of plant-insect mutualisms. *New Phytol* 172:412–428.
20. 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.
21. Weber MG, Keeler KH (2013) The phylogenetic distribution of extrafloral nectaries in plants. *Ann Bot* 111:1251–1261.
22. 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.
23. Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR (2010) Convergent evolution of seed dispersal by ants, and phylogeny and biogeography in flowering plants: A global survey. *Perspect Plant Ecol Evol Syst* 12:43–55.
24. Turner KM, Frederickson ME (2013) Signals can trump rewards in attracting seed-dispersing ants. *PLoS One* 8:e71871.
25. Trager MD, et al. (2010) Benefits for plants in ant-plant protective mutualisms: A meta-analysis. *PLoS One* 5:e14308.
26. Davidson DW, McKey D (1993) The evolutionary ecology of symbiotic ant-plant relationships. *J Hymenopt Res* 2:13–83.
27. Gaume L, McKey D, Terrin S (1998) Ant–plant–homopteran mutualism: How the third partner affects the interaction between a plant-specialist ant and its myrmecophyte host. *Proc R Soc Lond B Biol Sci* 265:569–575.
28. Stadler B, Dixon T (2008) *Mutualism* (Cambridge Univ Press, Cambridge, UK).
29. Weber MG, Agrawal AA (2014) Defense mutualisms enhance plant diversification. *Proc Natl Acad Sci USA* 111:16442–16447.
30. Pemberton RW (1992) Fossil extrafloral nectaries, evidence for the ant-guard anti-herbivore defense in an Oligocene *Populus*. *Am J Bot* 79:1242–1246.
31. Dlussky GM (1997) Genera of ants (Hymenoptera: Formicidae) from Baltic amber. *J Paleontol* 31:616–627.
32. 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.
33. Muschner VC, Zamberlan PM, Bonatto SL, Freitas LB (2012) Phylogeny, biogeography and divergence times in *Passiflora* (Passifloraceae). *Genet Mol Biol* 35(Suppl 1): 1036–1043.
34. Spriggs EL, et al. (2015) Temperate radiations and dying embers of a tropical past: The diversification of *Viburnum*. *New Phytol* 207:340–354.
35. Dunn RR, Gove A, Barrachlough TG, Givnish TJ, Majer JD (2007) Convergent evolution of an ant-plant mutualism across plant families, continents and time. *Evol Ecol Res* 9: 1349–1362.
36. Hinchliff CE, Smith SA (2014) Some limitations of public sequence data for phylogenetic inference (in plants). *PLoS One* 9:e98986.
37. Fritz SA, Purvis A (2010) Selectivity in mammalian extinction risk and threat types: A new measure of phylogenetic signal strength in binary traits. *Conserv Biol* 24: 1042–1051.
38. Garland T, Dickerman AW, Janis CM, Jones JA (1993) Phylogenetic analysis of covariance by computer simulation. *Syst Biol* 42:265–292.
39. Rabosky DL, Huang H (2016) A robust semi-parametric test for detecting trait-dependent diversification. *Syst Biol* 65:181–193.
40. Maddison WP, FitzJohn RG (2015) The unsolved challenge to phylogenetic correlation tests for categorical characters. *Syst Biol* 64:127–136.
41. Staab M, Fornoff F, Klein A-M, Blüthgen N (2017) Ants at plant wounds: A little-known trophic interaction with evolutionary implications for ant-plant interactions. *Am Nat* 190:442–450.
42. Delabie JHC (2001) Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): An overview. *Neotrop Entomol* 30:501–516.
43. Stadler B, Dixon AFG (1999) Ant attendance in aphids: Why different degrees of myrmecophily? *Ecol Entomol* 24:363–369.
44. Vea IM, Grimaldi DA (2016) Putting scales into evolutionary time: The divergence of major scale insect lineages (Hemiptera) predates the radiation of modern angiosperm hosts. *Sci Rep* 6:23487.
45. Oliver TH, Leather SR, Cook JM (2008) Macroevolutionary patterns in the origin of mutualisms involving ants. *J Evol Biol* 21:1597–1608.
46. Bass M, Cherrett JM (1995) Fungal hyphae as a source of nutrients for the leaf-cutting ant *Atta sexdens*. *Physiol Entomol* 20:1–6.
47. Jolivet P (1998) *Interrelationship Between Insects and Plants* (CRC Press, Boca Raton, FL).
48. Robson SK, Kohout RJ (2007) A review of the nesting habits and socioecology of the ant genus *Polyrhachis* Fr. Smith. *Asian Myrmecol* 1:81–99.
49. Blüthgen N, Feldhaar H (2009) Food and shelter: How resources influence ant ecology. *Ant Ecology*, eds Lach L, Parr CL, Abbott KL (Oxford Univ Press, Oxford), pp 115–136.
50. Ward PS, Branstetter MG (2017) The acacia ants revisited: Convergent evolution and biogeographic context in an iconic ant/plant mutualism. *Proc Biol Sci* 284:20162569.
51. Benson WW (1985) Amazon ant-plants. *Amazonia*, eds Prance GT, Lovejoy TE (Pergamon Press, Oxford), pp 239–266.
52. Ward PS (1991) Phylogenetic analysis of pseudomyrmecine ants associated with domatia-bearing plants. *Ant-Plant Interactions*, eds Huxley CR, Cutler DF (Oxford Science Publishers, Oxford), pp 335–352.
53. Jablonski D (2008) Biotic interactions and macroevolution: Extensions and mismatches across scales and levels. *Evolution* 62:715–739.
54. Lanfear R, Calcott B, Ho SYW, Guindon S (2012) Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol* 29:1695–1701.
55. Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)* (Institute of Electrical and Electronics Engineers, Piscataway, NJ).
56. Barden P (2017) Fossil ants (Hymenoptera: Formicidae): Ancient diversity and the rise of modern lineages. *Myrmecol News* 24:1–30.
57. Brady SG, Schultz TR, Fisher BL, Ward PS (2006) Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proc Natl Acad Sci USA* 103: 18172–18177.
58. Smith SA, O'Meara BC (2012) treePL: Divergence time estimation using penalized likelihood for large phylogenies. *Bioinformatics* 28:2689–2690.
59. Rabosky DL (2014) Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS One* 9:e89543.
60. Beaulieu JM, O'Meara BC, Donoghue MJ (2013) Identifying hidden rate changes in the evolution of a binary morphological character: The evolution of plant habit in campanulid angiosperms. *Syst Biol* 62:725–737.
61. Beaulieu JM, Donoghue MJ (2013) Fruit evolution and diversification in campanulid angiosperms. *Evolution* 67:3132–3144.
62. Pagel M (1994) Detecting correlated evolution on phylogenies: A general method for the comparative analysis of discrete characters. *Proc R Soc Lond B Biol Sci* 255:37–45.
63. Beaulieu JM, O'Meara BC (2016) Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Syst Biol* 65:583–601.
64. Stamatakis A, Aberer AJ (2013) Novel parallelization schemes for large-scale likelihood-based phylogenetic inference. 2013 IEEE 27th International Symposium on Parallel and Distributed Processing (Institute of Electrical and Electronics Engineers, Piscataway, NJ), pp 1195–1204.
65. 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. *Am J Bot* 100:556–573.
66. Weber MG, Porturas LD, Keeler KH (2015) World list of plants with extrafloral nectaries. Available at www.extrafloralnectaries.org/. Accessed April 25, 2017.
67. Orme D, et al. (2013) Caper: Comparative analyses of phylogenetics and evolution in R. Version 1.0.1. Available at https://cran.r-project.org/web/packages/caper/index.html. Accessed January 22, 2018.