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Evolutionary déjà vu? A case of convergent evolution in an ant–plant association

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Obligatory ant–plant symbioses often appear to be single evolutionary shifts within particular ant lineages; however, convergence can be revealed once natural history observations are complemented with molecular phylogenetics. Here, we describe a remarkable example of convergent evolution in an ant–plant symbiotic system. Exclusively arboreal, *Myrmelachista* species can be generalized opportunists nesting in several plant species or obligately symbiotic, live-stem nesters of a narrow set of plant species. Instances of specialization within *Myrmelachista* are known from northern South America and throughout Middle America. In Middle America, a diverse radiation of specialists occupies understory treelets of lowland rainforests. The morphological and behavioural uniformity of specialists suggests that they form a monophyletic assemblage, diversifying after a single origin of specialization. Using ultraconserved element phylogenomics and ancestral state reconstructions, we show that shifts from opportunistic to obligately symbiotic evolved independently in South and Middle America. Furthermore, our analyses support a remarkable case of convergence within the Middle American radiation, with two independently evolved specialist clades, arising nearly simultaneously from putative opportunistic ancestors during the late Pliocene. This repeated evolution of a complex phenotype suggests similar mechanisms behind trait shifts from opportunists to specialists, generating further questions about the selective forces driving specialization.

1. Introduction

In multispecies interactions, ecological circumstances may select for similar evolutionary responses in diverse species, resulting in recurrent convergent evolution [1]. Although convergent adaptation can be demonstrated by identifying functionally relevant phenotypes in different species, phylogenetic information improves our ability to make inferences about convergent evolution. A prominent example of such functionally convergent phenotype is found in the association between ants and plants [2–4], whereby many ant lineages have independently evolved specialized arboreal nesting habits [4,5]. Obvious instances of convergent evolution among ant–plant interactions can be identified through morphological and behavioural traits. Plants bearing domatia, extrafloral nectaries and food bodies have evolved independently multiple times in association with distantly related ants [3,4]. Once interdependencies between symbionts have been established, subsequent diversification within lineages, ant or plant, can lead to multiple species exhibiting symbiotic associations resulting from shared ancestry rather than convergence. Thus, morphologically and behaviourally similar sets of ant specialists, e.g. multiple species in the same closely related lineages, are assumed to result from a single evolutionary transition to specialization (e.g. within certain

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clades of *Cecropia*-symbiotic *Azteca* [6], *Vachellia*-inhabiting *Pseudomyrmex* [7]). In one case, however, recent studies have revealed an unexpected instance of convergence among closely related plant–ant species. The mutualistic ants associated with the iconic *Pseudomyrmex–Vachellia* system were discovered to comprise two clades separated by non-mutualistic species [8,9]. This independent evolution of two clades of *Vachellia* ants is striking because the specialized nest site selection and aggressive defence of nest space by these two *Pseudomyrmex* subgroups are lacking in the generalist species that separate them [9]. In this case, the two clades were morphologically distinguishable, but still considered to be sister clades prior to the molecular results [7]. Given this finding, other examples of convergence among closely related plant–ant species may exist in nature, but to reveal such cases, it is necessary to integrate natural history information with a robust molecular phylogenetic hypothesis [1,3,8,9].

Here, we report an even more extreme example of convergent evolution in an ant–plant system. The formicine genus *Myrmelachista* is a Neotropical lineage of exclusively arboreal ants. *Myrmelachista* and its sister taxon *Brachymyrmex* form a clade sister to all other formicine ants, and this clade is estimated to have originated in the Neotropics between 72.2 and 80 Ma [10]. Currently, 56 species and 13 subspecies of *Myrmelachista* are recognized [11], with species occurring from central Mexico to southern Argentina. The species exhibit a full range of arboreal nesting habits that we separate into two broad categories: (i) dead-stem nesters with generalized foragers [12,13] and live-stem nesters opportunistically nesting in a variety of plant species [12–14] or (ii) specialized live-stem nesters (=obligately symbiotic), nesting only in live stems of a narrow set of plant species, with most individuals of the plant species inhabited by the specialist ants [13,15–19] (figure 1). Nesting in dead stems and nesting opportunistically in live stems appear to be part of a continuous trait space, and some species can be found nesting in either dead or live stems. In contrast, the ant–plant specialists are sharply differentiated in their distinct use of a restricted set of plant species (figure 1).

In *Myrmelachista*, the known specialized associations occur in two regions. In the northern portion of Amazonia in South America, *M. schumanni* is a specialist inhabitant of *Duroia hirsuta* (Rubiaceae) and *Tococa* (Melastomataceae), systematically poisoning all plants in the vicinity and creating monodominant vegetation patches called 'devil's gardens' [16,17,20,21]. In the devil's garden system, the host plants have conspicuous preformed domatia. In Middle America, seven species have specialized associations with small-sized plants of *Mespilodaphne*, *Ocotea* (Lauraceae) and *Guarea* (Meliaceae) dispersed in the understory [13,15,19]. The host plants involved in the Middle American associations lack conspicuous domatia, or if present, they are subtle [19]. One plant trait that correlates with the Middle American association is dwarfism; all associated plants are treelets that flower and fruit in the understory or forest gaps, while most of their congeners are large canopy trees. In neither of the specialized associations is there evidence for food bodies or extrafloral nectaries. Both associations are thought to be mutualisms [15,16,18,19,22], with either evidence or speculation that the ants: (i) provide defence against herbivores [15,16], (ii) remove spores, lichens, epiphylls and debris from leaf surfaces [18,19], (iii) protect from encroaching plants [16,17,21] and (iv) provide beneficial nutrients from food items brought into stems [22]. In return, the host plant provides living space for the ants.

Regarding the ant partners, the opportunist and specialist species of the Middle American radiation of *Myrmelachista* display a clear phenotypic distinction in worker colour, worker behaviour and queen behaviour. The worker caste of opportunist species varies in colour from black to dusky orange-brown, with the exception of a poorly known species that has orange workers (*M. meganaranja* Longino, 2006) [13]. In contrast, all specialist species have light orange to yellow workers (electronic supplementary material, figure S1). In addition, the specialist species show a high degree of morphological uniformity, with workers of different species essentially indistinguishable. With respect to worker behaviour, the opportunist species tend to occupy only a part of the host plant and lack patrolling behaviour. In contrast, specialist *Myrmelachista* occupy the entire host plant and often patrol new growth surfaces. The specialist species often harbour hemipteran partners (i.e. coccids and pseudococcids) in unique xylem pockets that extend from the nest lumen to the cambium. We have not observed these pockets in the nests of opportunist species. Finally, colony-founding queens of opportunist species presumably have relatively generalized searching behaviours, selecting a variety of plant cavities. Foundresses of specialists restrict their searching to particular species of host plants.

For Middle American *Myrmelachista*, where there are multiple, similar-looking specialist species present, it has generally been thought these species form a clade and that specialization arose once in the common ancestor [13]. This hypothesis is supported by the strong morphological and behavioural uniformity of specialists. However, there has never been an explicit phylogenetic reconstruction of the genus, and relationships among species remain speculative. In this study, we explore the evolution of nesting behaviour and this unique plant–ant specialization in *Myrmelachista* for the first time. We employ a phylogenomic approach using ultraconserved element (UCE) sequence capture [23,24] to estimate the phylogenetic relationships within the genus. Our taxon sampling covers the full morphological, behavioural and geographical range of *Myrmelachista*, with a particularly comprehensive sampling of the Middle American species. Our initial goal was to examine whether the South American and Middle American cases of myrmecophytism were the result of a single evolutionary specialization or independent events. We discovered not only the independent evolution of plant specialists in South and Middle America but also the unexpected result that the Middle American specialists were two independent clades, separated by generalist ancestors and arising nearly simultaneously.

2. Results

(a) Phylogeny and divergence times of *Myrmelachista*

Using a dataset comprised 1946 UCE loci and 45 terminals, we recovered a robust phylogeny of *Myrmelachista* ants and found little topological conflict across analyses (figure 2; electronic supplementary material, figures S2–S8). We found strong

Figure 1. Nest detail and habitus of *Myrmelachista*. (*a*) Opportunist species (*Myrmelachista zeledoni* Emery, 1896) and (*b*) specialist species (*Myrmelachista lauropacifica* Longino, 2006). Figures are not to scale.

support for five main clades: a Patagonian clade, two Neotropical clades, a Devil's Garden clade and a Middle American clade. The Patagonian clade is sister to all remaining *Myrmelachista*. It is restricted to the southern portion of South America and contains several species. Those species whose natural history is known are opportunistic nesters. Neotropical clade 1 and Neotropical clade 2 are found throughout the Neotropics and comprise a diverse assemblage of opportunistic nesters. The Devil's Garden clade occurs in northern South America, well separated from the Middle American specialists, and contains *M. schumanni*. This clade may contain only the single-species *M. schumanni* or a complex of closely related species, all of which are devil's garden ants (electronic supplementary material, table S1). Finally, the Middle American radiation is a large clade that occurs in northwest South America, the Caribbean and throughout the Middle American Corridor (from Panama to Mexico). Unexpectedly, specialist plant–ant species in the Middle American clade were found to occur as two independent clades separated by opportunists (figure 2). Although partially overlapping in range, one specialist clade is centred on the Caribbean slope, from Mexico to Costa Rica (hereafter Caribbean clade), and the other specialist clade is centred on the Pacific slope of Costa Rica (hereafter Pacific clade). A constraint analysis comparing the main topology (figure 2) to a tree in which the two specialist clades were forced to be monophyletic firmly rejected the alternative hypothesis (p-value of approximately unbiased (AU) test AU = 0.00137; electronic supplementary material, table S2).

Divergence dating analyses revealed that crown-group *Myrmelachista* evolved approximately 29 Ma (95% highest posterior density (HPD) 21.35–37.71 Myr) during the late-Oligocene (figure 3). The Middle American radiation evolved approximately 12 Ma (HPD 9.52–16.67 Myr). The three specialist clades were estimated to be reasonably young and similar in age, originating in the Pliocene. The crown-group ages were 3.83 Ma (HPD 2.61–5.30 Myr) for the Devil's Garden clade, 3.02 Ma (HPD 2.18–3.97 Myr) for the Caribbean clade and 3.32 Ma (HPD 2.44–4.30 Myr) for the Pacific clade of the Middle American specialists.

(b) Evolution of specialization

We examined the evolution of nesting phenotype by performing multiple ancestral state reconstructions (ASR) analyses using maximum parsimony (MP), maximum likelihood (ML) and Bayesian approaches. Analyses overwhelmingly supported the independent evolution of at least three instances of live-stem specialization from opportunist ancestors (figure 3; electronic supplementary material, tables S3–S5).

The MP analyses recovered specialization occurring independently in the Caribbean and Pacific clades as the most parsimonious scenario with different models returning the shortest total transition cost by either recovering as or having

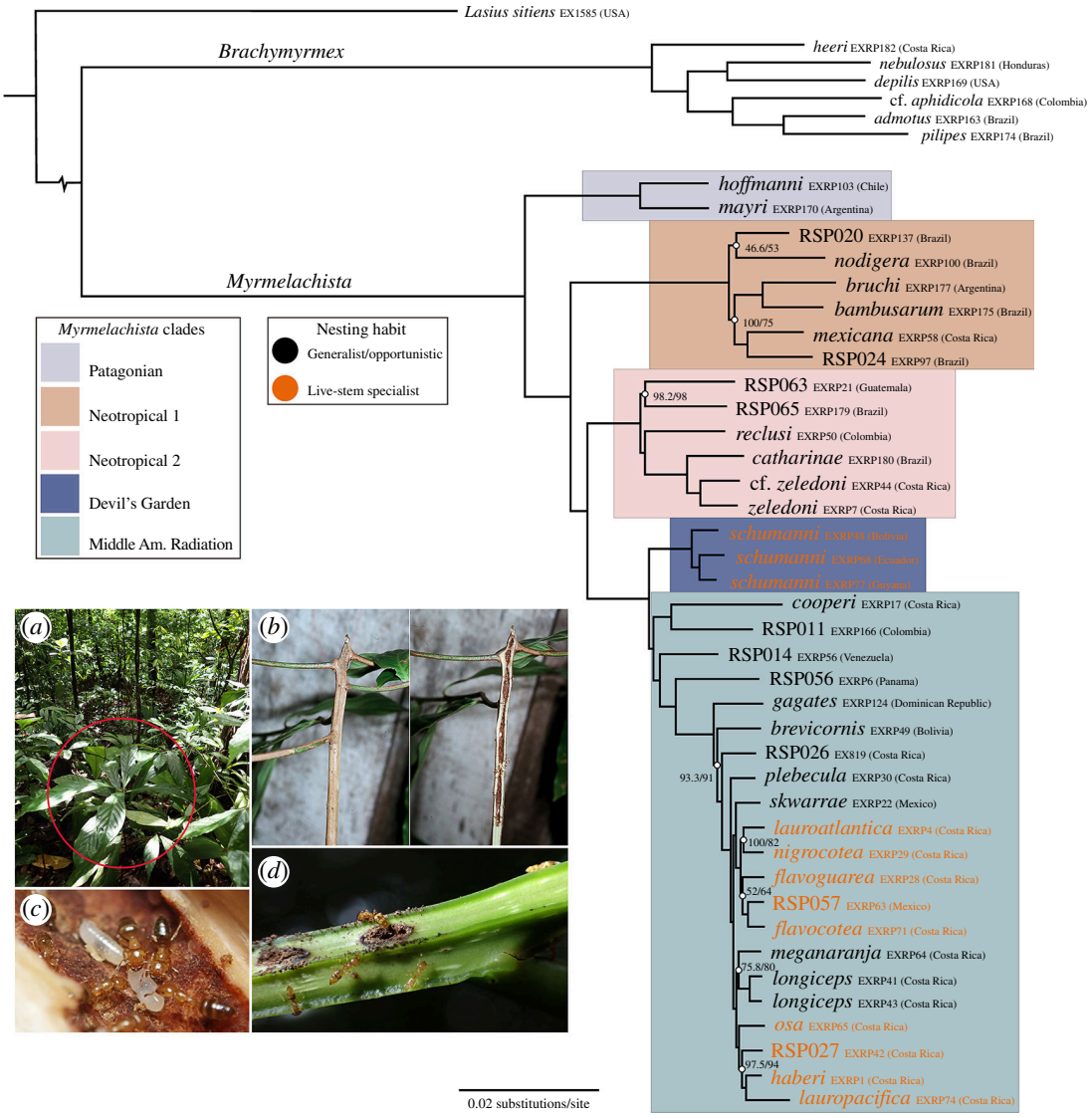

Figure 2. Phylogeny of *Myrmelachista* showing the major clades and instances of obligatory symbionts. Support values on nodes indicate ultrafast bootstrap (UFB) and Shimodaira-Hasegawa-like approximate likelihood ratio test scores (SH-aLRT) and are only shown for nodes not maximally supported by both measures. Clades are colour-coded based on the legend. Taxon names are colour-coded based on nesting habits and include a unique DNA extraction code for each sample and country where the specimen was collected. Features of specialist symbionts are shown in the inset: (*a*) understory of Costa Rican rainforest, red circle highlighting individual of *Mespilodaphne* sp. (Lauraceae host plant); (*b*) *M. lauropacifica*: detail of host branch exterior (left) and excavated interior completely occupied by workers (right); (*c*). *M. flavoguarea* workers tending larvae inside the nest gallery; (*d*) detail of excavated cavities outside of a *M. osa* nest.

M. cooperi coded as an opportunist species (for the hidden model) (electronic supplementary material, table S3 and figure S10).

For ML analyses, the ARD (all rates different among state transitions) models favoured the ancestor for the Caribbean and Pacific clades likely being a specialist (i.e. reversal favoured over convergence) (electronic supplementary material, table S3 and figures S11–S13). Similar log-likelihood values and AIC (Akaike information criterion) scores for the ML analyses were obtained with *M. cooperi* coded as opportunist and the models with equal rates among state transitions (ER) (recovering independent evolution of Middle American specialists) and ARD (electronic supplementary material, tables S4 and S5). However, the ER model was preferred over the ARD model after a log-likelihood ratio test ($p = 0.04$; electronic supplementary material, table S4).

Our reversible-jump Markov Chain Monte Carlo (rjMCMC) analyses also supported the scenario of independently evolved specialists. Interestingly, outputs recovered the ancestor of the Pacific and Caribbean clades likely being a specialist, supporting a scenario of reversal to the opportunist condition for the ancestor of the Pacific clade and subsequent regaining of the specialist condition for one Pacific subclade (containing *M. osa*, *M. haberi*, *M. lauropacifica* and *M.* RSP027, a new specialist species from Costa Rica) (electronic supplementary material, tables S3 and S6 and figure S14).

Owing to its peculiar morphology and natural history, additional experiments could confirm *M. cooperi* as a social parasite of specialist *Myrmelachista*. Similar results from our ASR analyses for all methods were obtained when *M. cooperi* was either coded as opportunist or unknown for nesting habit (electronic supplementary material, table S3). Overall, our results indicate that the Middle American *Myrmelachista* clade radiated rapidly (figure 3) and generated not only opportunist species but also two likely independent clades of remarkably similar specialist species that arose nearly simultaneously—and perhaps allopatrically—on the Caribbean and Pacific sides of Middle America.

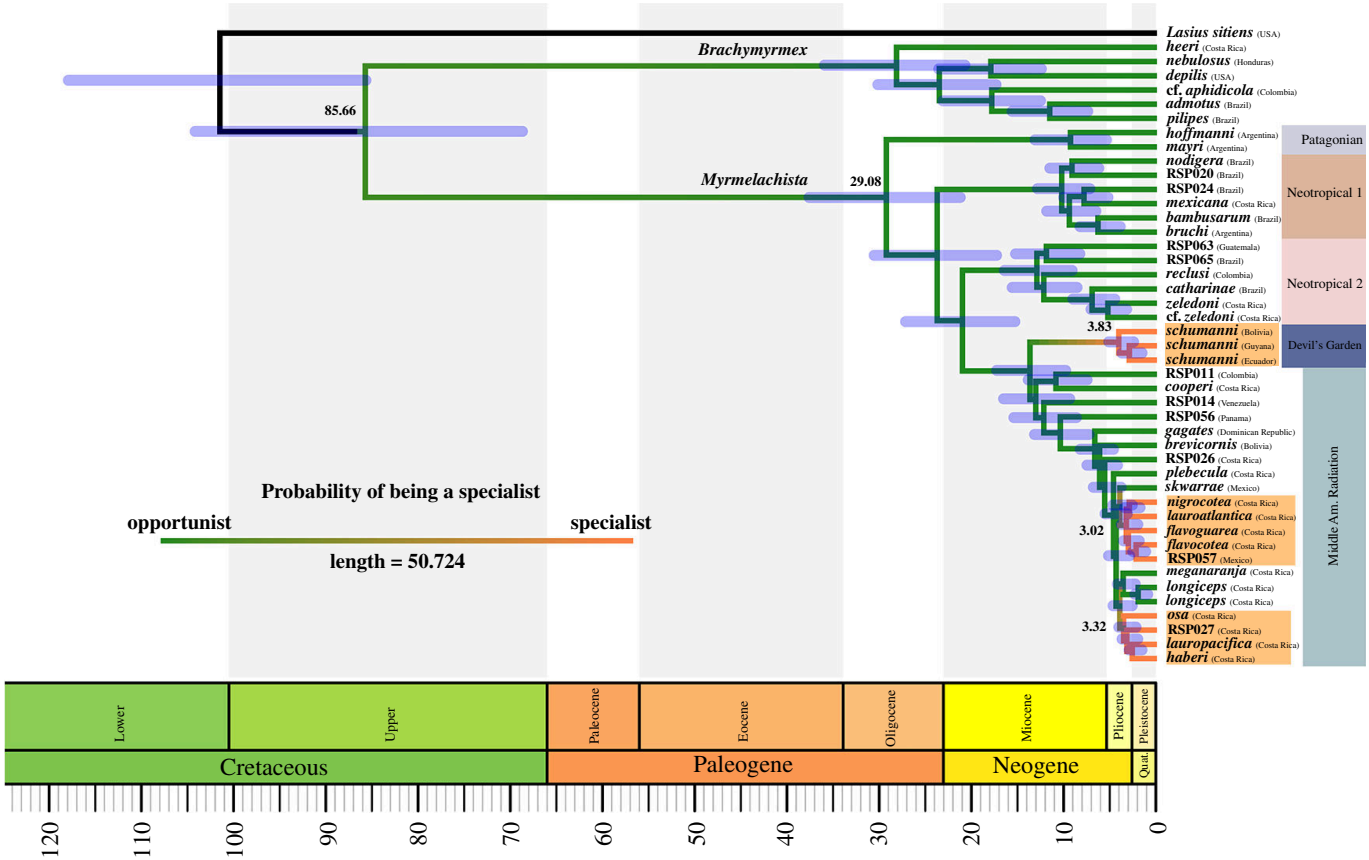

Figure 3. Divergence times and evolution of nesting specialization of *Myrmelachista*. Numbers above nodes are mean ages in millions of years, blue bars indicate 95% HPD. Nesting habit was classified as a discrete binary character for the ancestral state reconstruction (ASR), with species either being opportunistic or specialist (=obligate symbiont). Topology shows transitions of nesting habit for *Myrmelachista* using a maximum-likelihood reconstruction and a stochastic character mapping approach (*simmap* function in the R package 'phytools') and the equal rates (ER) model. Transitions of character states are shown as a summary of the reconstructed changes of 1000 stochastic character histories (green and orange tones). Specialist clades are coloured based on nesting habits, as indicated in figure 2; clades are colour-coded based on the legend from figure 2.

3. Discussion

(a) Evolutionary history of *Myrmelachista* specialists

We recovered a robust phylogenetic hypothesis for *Myrmelachista*, with most nodes receiving maximum support and, more importantly, exhibiting little topological conflict across analyses. We found that specialized ant–plant associations occurred independently at least three times in the genus. The South American devil's garden ants are distantly related to the other specialists and thus clearly evolved independently from the Middle American specialist species—plus, devil's garden species are phenotypically distinct from the other specialists (e.g. worker coloration and overall body shape, antennomere count for female castes, host plant use). For the Middle American specialists, we discovered that what was hypothesized to be a single radiation of ant–plant mutualists is actually two independent evolutionary events, occurring nearly simultaneously and with strikingly parallel morphological and behavioural features. One specialist clade is restricted to the Pacific slope of Costa Rica. The other specialist clade occurs mainly on the Caribbean slope, from southern Mexico to Costa Rica. Most of the Caribbean clade's diversity is in Costa Rica, with the species found in southern Mexico representing a relatively recent split from one of the Costa Rican species. This pattern suggests that both specialist clades originated in Costa Rica and the Caribbean clade later dispersed northwards.

(b) Nature of the mutualism

The evolution of Middle American *Myrmelachista* specialists is comparable to the *Pseudomyrmex–Vachellia* system [9], which also involves convergent evolution of host plant specialization. Both systems are relatively young, but differ in the degree of morphological divergence, the timing of events and the degree of sympatry. The two acacia ant clades are morphologically distinguishable, while the two *Myrmelachista* clades are not. The evolution of the two acacia ant clades was not simultaneous. One lineage arose in the Late Miocene in northern Middle America, and the other lineage probably originated in southern Middle America about 3 Myr later, apparently colonizing the pre-existing mutualism [9]. In contrast, the *Myrmelachista* clades arose simultaneously. The two *Pseudomyrmex* lineages are largely sympatric, while the two *Myrmelachista* clades are mostly non-overlapping, with only a small zone of contact. The one exception is morphospecies *Myrmelachista* RSP027 (a Pacific clade

species inhabiting the lower cloud forest envelope that can be found all the way to the Caribbean slope of the Cordillera Volcánica Central).

There are some similarities between the two systems. Both systems appeared to have evolved in the context of niche shifts. For *Pseudomyrmex*, the mutualism was initiated with a shift from closed to open habitats, where plants were more prone to herbivore pressure [9]. For Middle American *Myrmelachista*, the niche shift was probably linked to elevational changes. The opportunist relatives of the specialist clades are found in montane rainforests and cloud forests. The specialists are restricted to middle and lower elevations. The evolution of mutualistic *Myrmelachista* may be linked to downslope colonization in a lowland environment where the generalist arboreal niche is already packed with hostile arboreal ant competitors (e.g. *Crematogaster*, *Azteca*, *Camponotus*). The acacia system was a move to a high-energy system, requiring highly energetic rewards for conspicuous and pugnacious ants. The *Myrmelachista* system was the opposite, moving to a low-energy system and remaining 'under the radar' to other arboreal ants, with few overt rewards and relatively inconspicuous ant defenders. The extreme uniformity of traits across the mutualistic instances in the *Myrmelachista* system, an evolutionary déjà vu, may indicate that there are limited, almost deterministic solutions to the challenges posed by the environment.

(c) The host plants

Host plants in prominent examples of ant–plant symbioses typically bear prominent traits to attract ant partners [25], such as conspicuous preformed domatia and extrafloral nectaries [5]. These are lacking in the *Myrmelachista* system, or at most, there is a slight swelling of shoot tips that facilitates colonization [18,19]. The factors predisposing the development of mutualism are thus unclear. However, some plant traits correlate with the mutualism, even among unrelated plant hosts. One of the most prominent is dwarfism. Host plants of specialist *Myrmelachista* are small treelets, rarely more than 4 m tall, flowering and fruiting in the understory of small forest gaps. In contrast, the congeners not involved in symbiotic interactions with *Myrmelachista* are all tall canopy trees. This pattern holds for hosts in the Lauraceae (*Mespilodaphne* and *Ocotea*) and Meliaceae (*Guarea*). Dwarfism may have evolved independently of ant presence and been a pre-existing resource for ant colonization, or dwarfism might result from the mutualistic interaction. A potential dissolution of the mutualism might occur when host plants achieve a certain size, and plant growth might need to match colony growth to maintain the interaction [19]. Therefore, plants may thrive by limiting the nest space ants can occupy and effectively protect. Another potential explanation is that ants act as stressors for plant development, directly preventing growth. Being in a small understory treelet may have facilitated the radiation of specialist *Myrmelachista*. A second characteristic that *Myrmelachista* host plants share is a thin cortical layer and soft pith, traits favouring entry by stem-nesting ants [26]. Similar traits are observed in other mutualistic systems involving formicine ants (*Cladomyrma* in Southeast Asia [27]; *Petalomyrmex* in Africa [28]). In those systems involving other formicines, plant partners seem to be recent lineages [5]. Divergence timing estimates for the host plants in the *Myrmelachista* system are yet to be explored. However, molecular evidence shows that *Guarea tafae-malekui* (host for *Myrmelachista flavoguarea*) is confined to Costa Rica and has arisen recently from the South American *Guarea* stock [29] and that the genus *Mespilodaphne* is a short-branched lineage within the *Ocotea* complex [30].

(d) Convergence of the specialist phenotype in Middle America

Throughout Costa Rica in the wet forest understory, one will find one or more species of host treelets, nearly 100% of whose stems will be occupied by tiny yellow ants. These ants look the same and act the same. Longino distinguished seven species based on queen colour, size and head shape [13]. Worker differences are extremely subtle to non-existent. Thus, to find these species divided among two independent clades in our UCE phylogeny was completely unexpected and begs the question 'what factors, both ecological and genomic, could produce such extreme similarity between two independent clades'? A potential explanation for the convergence itself is that the evolutionary pathway from opportunist to specialist is deterministic, with the evolution of host plant specialization being a similar outcome. Deep homology (i.e. the homology of complex regulatory circuitry inherited from a common ancestor) [31,32] may be providing the foundation for independently evolved traits, such as loss of integumental pigmentation in workers and host-searching behaviour in founding queens.

However, the deterministic nature of specialization alone cannot explain the convergence in the timing of the origination of the two clades of specialists in the Middle American radiation. Therefore, one could speculate that the presence of common selective agents precipitating specialization occurred simultaneously on the two slopes of Costa Rica, e.g. environmental factors, plant host availability and niche competition. It is possible that simultaneous ecological conditions for mutualism may occur in different regions within the range of *Myrmelachista*, such that a specialist niche is frequently available but may be dynamic, shifting over space and time. A combination of precursor material for convergence and constrained pathways to mutualism may allow specialists to evolve repeatedly, similarly filling the niche each time it appears [33].

Another explanation is that occasional introgression with opportunist species could transport the genomic pieces linked to the specific lifestyle across different lineages, similar to what is observed with *Heliconius* butterflies, in which genes for adaptive wing patterns cross species boundaries [34,35]. In this scenario, rather than a sporadic gene exchange, specialist species of *Myrmelachista* could form an admixed 'population', with mass hybridization generating the persistence of the specialist phenotype. For pairs of closely related species, we might expect the repeatability of genomic evolution after hybridization events [36,37]. The genomic landscape (i.e. composition, conserved recombination maps, shared mechanisms of selection) of specialist *Myrmelachista* could, therefore, allow for the specialist phenotype to be 'floating' above species boundaries.

Additionally, young clades might experience comparatively faster speciation and extinction rates [38]. The specialization in *Myrmelachista* could be an evolutionary dead end, frequently occurring but rapidly going extinct—such that there may

have been a long history of specialists stretching far back in time. While specialization in narrow ecological niches may allow species to survive in competitive or unique environments (e.g. [39,40]), it may also imply that specialized taxa may be doomed to extinction before they can transition to a more stable state over evolutionary time [41]. The growing empirical evidence available from clades with detailed phylogenetic and ecological information suggests contrasting outcomes, with niche specialization pushing clades towards the edge of extinction (e.g. [42,43]) or not (e.g. [40,44,45]).

One ASR (electronic supplementary material, figure S12) favoured reversal over convergence. Instead of recent convergence, a single old lineage of specialists may have experienced multiple instances of reversal to the niche of opportunist nesting (see electronic supplementary material, tables S3–S5 and figures S11–S14). Are convergence of specialization and numerous reversals to the opportunistic condition equally probable? Although our results support the former, in processes similar to those described above, specialist species could harbour older genetic elements that allow the reappearance of opportunist lineages. This is similar to what was recently observed in fruit flies, with hybrids rapidly regressing to one of the parental species, becoming nearly indistinguishable in morphology and behaviour [46]. The loss of specialization in *Myrmelachista*, therefore, could be linked with an apparent opportunist phenotype having evolved mechanisms to inhibit specialization.

(e) Conclusions

While it is well established that ant–plant mutualisms have evolved multiple times independently across different ant lineages, it is uncommon to find several originations of the behaviour within individual ant genera. Our results reveal that plant–ant specialization has evolved at least three times in the unique *Myrmelachista* system. Furthermore, this 'under the radar' system includes an instance of remarkable convergent evolution. The déjà vu within Middle American *Myrmelachista* shows that independently evolved ant–plant mutualisms can be strikingly similar in tempo and mode of evolution. The cryptic nature of the *Myrmelachista*–plant mutualisms in the Middle American clade makes the study of its symbiotic origins challenging, but a comparison of independent origins could reveal the degree of convergence versus divergence in the underpinning mechanisms. Questions about the selective forces driving and maintaining these symbiotic interactions are open for further studies. Therefore, exploring the genomic signatures of ant–plant mutualistic interactions should be the next step, revealing mechanisms of selection and functional space [47].

4. Methods

(a) Resource availability

(i) Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Rodolfo da Silva Probst (probstrodolfo@gmail.com).

(ii) Data, code and materials

Raw Illumina reads and contigs representing UCE loci have been deposited at Dryad (https://doi.org/10.5061/dryad.4j0zpc8m9) [48]. A complete list of NCBI (National Center for Biotechonology Information) accession numbers and further details of samples and voucher specimens can also be accessed at Dryad, along with all UCE matrices, Trinity contigs, tree files, unfiltered UCE alignments and additional data analysis files (partitioning schemes and log files) [48]. The Phyluce package and associated programs can be downloaded from https://www.ultraconserved.org/#software or its GitHub repository [49]. The ant-specific baits used for UCE enrichments are available from Arbor Biosciences (arborbiosci.com/genomics/targeted-sequencing/mybaits/mybaits-expert/mybaits-expert-uce/). The UCE bait sequence file is available at Figshare (https://figshare.com/articles/dataset/Hymenoptera_UCE_and_Exon_bait_sets_from_Branstetter_et_al_2017_/4630375/1).

(b) Method details

(i) Taxon sampling and phylogeny

Our sample set comprised 45 specimens. Thirty-eight were species of *Myrmelachista*: one from the Caribbean, 21 from Middle America and 16 from South America. The remaining seven specimens were outgroup taxa, selected based on a phylogeny of the Formicinae by Blaimer *et al*. [10]. We employed a phylogenomic approach, combining target enrichment of UCEs with multiplexed, next-generation sequencing [23,24]. All molecular work was performed following a phylogenomic pipeline combining target enrichment of UCEs with multiplexed, next-generation sequencing described in Branstetter *et al.* [24] and included DNA extraction, library preparation, UCE enrichment and sample pooling. Sequencing was performed on an Illumina HiSeq 2500 at the University of Utah genomics core facility or on a HiSeq X at Novogene Corporation, Inc. For UCE enrichment, we used an ant-customized bait set ('ant-specific hym-v2') targeting 2524 UCE loci across Hymenoptera [24].

We carried out multiple phylogenetic reconstructions on the final matrix (1946 UCE loci for a 75% complete matrix) using the likelihood-based program IQ-Tree v2.0.4 [50] to explore the effects of data partitioning, including entropy-based

Sliding-Window Site Characteristics (SWSC-EN) partitioning [51], genealogical concordance, topological structure and sensitivity analyses (additional information in the electronic supplementary material). We used the coalescent program Astral-III [52] to account for gene tree discordance arising from independent lineage sorting while inferring a species tree. We inferred a dated time tree for *Myrmelachista* and its species using BEAST2 v2.6.3 [53] and MCMCTree v4.9j, part of the PAML package [54].

All the phylogenetic analyses were conducted using either the CIPRES platform [55] or the Center for High Performance Computing at the University of Utah.

(ii) Ancestral state reconstruction

In this study, we explored ASR for nesting habit for our *Myrmelachista* dataset using three distinct approaches and several different programs: MP [56], ML [56–58] and rjMCMC [59] (see electronic supplementary material, "Supplementary Text" for a complete description of methods). Ancestral trait estimation for nesting habit was based on known natural history data (collection data, specimen labels or literature information) for all *Myrmelachista* taxa included in our dataset. Terminals were trimmed to avoid duplicate specialists that could influence analyses outputs. We classified nesting habit as a discrete binary character: (0) opportunistic nester and (1) specialized live-stem nester. For the input tree, we selected the ML tree inferred using the SWSC-EN partitioning method owing to its improved sensibility to UCE mutation rates when compared with concatenated or partitioned by locus [51]. Therefore, results and interpretation are dependent on this specific topology—which could potentially extend to ASR outputs. For an extensive explanation of model approaches for each reconstruction analysis, please refer to the electronic supplemental material.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. Raw Illumina reads and contigs representing UCE loci have been deposited at Dryad [48]. A complete list of NCBI accession numbers and further details of samples and voucher specimens can also been accessed at Dryad, along with all UCE matrices, Trinity contigs, tree files, unfiltered UCE alignments and additional data analysis files (partitioning schemes and log files).

The Phyluce package and associated programs can be downloaded from https://www.ultraconserved.org/#software or its GitHub repository [49]. Please also see Phyluce original publication [60].

The ant-specific baits used for UCE enrichments are available from Arbor Biosciences (arborbiosci.com/genomics/targeted-sequencing/mybaits/mybaits-expert/mybaits-expert-uce/). The UCE bait sequence file is available at Figshare [61].

Supplementary material is available online [62].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. R.S.P.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, writing—original draft, writing—review and editing; J.T.L.: conceptualization, data curation, funding acquisition, investigation, resources, supervision, writing—original draft, writing—review and editing; M.G.B.: data curation, formal analysis, funding acquisition, investigation, methodology, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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