Defensive traits exhibit an evolutionary trade-off and drive diversification in ants

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Evolutionary biologists have long predicted that evolutionary trade-offs among traits should constrain morphological divergence and species diversification. However, this prediction has yet to be tested in a broad evolutionary context in many diverse clades, including ants. Here, we reconstruct an expanded ant phylogeny representing 82% of ant genera, compile a new family-wide trait database, and conduct various trait-based analyses to show that defensive traits in ants do exhibit an evolutionary trade-off. In particular, the use of a functional sting negatively correlates with a suite of other defensive traits including spines, large eye size, and large colony size. Furthermore, we find that several of the defensive traits that trade off with a sting are also positively correlated with each other and drive increased diversification, further suggesting that these traits form a defensive suite. Our results support the hypothesis that trade-offs in defensive traits significantly constrain trait evolution and influence species diversification in ants.

KEY WORDS: Ancestral state reconstruction, defense, evolutionary trade-off, Formicidae, trait-based diversification.

All species experience constraints arising from developmental, functional, and energetic limitations. These limitations have featured prominently in the development of ecological and evolutionary theories since Darwin (Darwin 1859), and include optimal foraging theory (Krebs 1978), key innovation theory (Crother et al. 2007), and life-history theory (Stearns 1989; Tilman 2011). One concept shared by these theories is the importance of tradeoffs between different investment strategies. Ecological theories on tradeoffs predict differential energy investment in different traits based on resource limitations or conflicting environmental demands (e.g., Werner and Anholt 1993). In contrast, evolutionary trade-off theory posits that energy investment in a given trait in response to some selective force limits investment in other traits that serve similar functions. Therefore, such traits should be negatively associated with each other across species over evolutionary time (Stearns 1989; Arnold 1992; Mooney et al. 2010; Tilman 2011; Ferenci 2016).

Trait trade-offs influence various evolutionary processes, including patterns of morphological divergence (DeWitt et al. 2000; Svanbäck and Eklöv 2003; Muchhala 2007; Holzman et al. 2012; Heinen-Kay et al. 2015) and rates of diversification (Schluter 1995; Holzman et al. 2012). For example, Muchhala (2007) found that increased specialization of floral morphology in *Burmeistera* is mediated by an adaptive trade-off arising from differential efficiency of bat and hummingbird pollination. Holzman et al. (2012) showed that biomechanical trade-offs in the feeding morphology of fishes promote elevated rates of evolution in the traits contributing to the trade-off. Trade-offs could ultimately limit rates of speciation or morphological evolution by restricting species to particular regions of morphospace or ecological niches (Shoval et al. 2012), as seen in guppies (Ghalambor et al. 2004) and bacteria (Ferenci 2016). However, others have suggested that trade-offs may promote diversification in some cases (Schluter 1995; de Vos et al. 2015). For example, Herrel et al. (2009) find evidence for a trade-off between bite force and jaw movement velocity in Darwin's finches, and suggest that this trade-off may lead to differences in the evolution of song production and, as a consequence, drive reproductive isolation and subsequent speciation.

In practice, it can be difficult to assess the role of trait trade-offs over evolutionary timescales. Determining the ecological function of focal traits is important, as a negative correlation between traits may not be the a priori expectation for sets of traits that are not functionally redundant (Agrawal 2011). Furthermore, the variability and diversity of processes acting on morphological and species diversification may influence evolutionary dynamics, leading to unexpected patterns (Bennett and Lenski 2007). However, despite these challenges, evidence for an evolutionary trade-off can be assessed in a system with labile traits of known or likely functions and a fairly complete and sizeable phylogeny (Maddison and FitzJohn 2015).

Over the past several decades, there has been much interest in ants as a group of insects that are highly influential in many terrestrial ecosystems (Hölldobler and Wilson 1990; Lach et al. 2010). In particular, recent work has significantly improved our understanding of phylogenetic relationships between ant species, genera, and subfamilies (Brady et al. 2006; Moreau et al. 2006; Lapolla et al. 2010; Ward et al. 2010; Moreau and Bell 2013; Schmidt 2013; Blaimer et al. 2015; Brady et al. 2015; Ward et al. 2015), providing a crucial foundation for understanding evolutionary processes. Furthermore, a growing body of research has focused on relationships between morphological traits and ecological niche in ants. Such eco-morphological relationships include eye size and diet (Weiser and Kaspari 2006), caste specialization and prey type (Powell and Franks 2006), and morphological specialization and latitude (Silva and Brandão 2014). Additionally, a few studies on single genera or geographically restricted communities have correlated aspects of morphological evolution with some specific evolutionary or ecological process such as ecological release or niche adaptation (Wilson 1959; Pie and Traniello 2007; Moreau 2008; Sarnat and Moreau 2011; Blaimer et al. 2015; Price et al. 2016). However, to date, relatively little work has integrated phylogenetic and eco-morphogical data to address trait-based diversification and evolutionary processes in ants, especially on a broad, family-wide scale.

Defensive traits are compelling candidate drivers of evolution across many taxa, including in ants (Hunt 1983), and may be strongly selected as an anti-predator defense. Predation has been invoked as an important selective force influencing clade diversification and morphological divergence across many taxa, including plants (Agrawal 2011; Weber and Agrawal 2014; Endara et al. 2015), amphibians (Arbuckle and Speed 2015), dragonflies (Mikolajewski et al. 2010), and beetles (Ge et al. 2011). In ants, traits thought to confer defensive benefits to a colony are remarkably varied across genera, including stings, spines, chemical defenses, recruitment and deployment of a soldier caste, and cryptic foraging strategies (Hunt 1983; Lach et al. 2010). These key traits are likely costly and under strong selection pressure, and should therefore exhibit some trade-off in energy investment. The diversity of defensive morphologies and life-history strategies suggests there may be an evolutionary trade-off among some of these traits. This hypothesis is corroborated by observational evidence of defensive trait trade-offs across subfamilies and genera (Moreau, pers. obs.), but the role of trait trade-offs in constraining or otherwise influencing evolution in ants has not yet been rigorously tested. In fact, most general patterns relating to ant morphological defense have not been investigated (Lach et al. 2010).

We reconstruct an expansive, genus-level phylogeny of ants that includes 268 described genera (82% of all described ant genera), compile a genus level database for eight morphological and ecological traits, and conduct trait-based diversification and phylogenetic correlation analyses to address whether defensive traits exhibit evolutionary trade-offs in ants. These data and analyses additionally allow us to investigate the evolutionary lability of multiple defensive traits and the impact of these traits on rates of diversification. Although functional traits are likely to play a significant role in ant evolution, the influence of these traits on patterns of diversification have rarely been investigated, and the processes constraining the evolution of these traits are still unknown. This study will therefore provide a foundation for future work on trait-based evolutionary processes in ants.

Methods
MOLECULAR DATA
We inferred a genus-level molecular phylogeny of the ants using sequences from previously published studies (Lapolla et al. 2010; Ward et al. 2010; Moreau and Bell 2013; Schmidt 2013; Brady et al. 2014; Ward et al. 2015) and 19 additional sequences from GenBank (Table S1). The sequences retrieved from Moreau and Bell (2013) are derived from three prior studies (Brady et al. 2006; Moreau et al. 2006; and Rabeling et al. 2008). From these sources, sequences were available for 648 ant species covering 268 described genera, as well as 18 outgroup taxa, spanning nine nuclear and mitochondrial genetic markers. For each species, all available genes were included even if derived from different individuals. In total, 45.2% of species had data for all nine genes, and 96.1% of species had data for four or more genes. Sequences were aligned using MUSCLE in R (Edgar 2004; R Core Team 2015). The alignment was then inspected visually, and ambiguously aligned sites were excluded manually in Mesquite v3.02 (Maddison and Maddison 2015). The final aligned data matrix was deposited in TreeBASE (URL: http://www.purl.org/phylo/treebase/phylows/study/TB2:S19313; http://www.treebase.org).

PHYLOGENETIC INFERENCE
An initial tree with 666 terminals was inferred under a maximum likelihood framework in RAxML v8 (Stamatakis 2014) on the

CIPRES computing cluster (Miller et al. 2010). The concatenated matrix of all nine genes was partitioned so that the GTR + Γ model of evolution for each gene was independent of each other. Support for inferred relationships was determined with bootstrapping, with the program automatically halting bootstrapping after a sufficient number of replicates, and the maximum likelihood tree was then pruned to include only one species per genus, to control for uneven genus sampling across the tree. Eighteen genera included in our study that are currently recognized in the literature are known to actually represent a polyphyletic grouping of species, but have yet to be formally revised. When this was the case, we either chose one species as the representative for the genus or retained multiple lineages with distinct identifying names (see Table S2 for a more detailed description of our selection process following the recent taxonomic literature). When we retained multiple tips for a genus (only four of our 268 described ant genera), we used the same trait states for the genus for all tips and evenly divided the number of species in the genus by the number of tips in our tree. In addition to 16 outgroup taxa from related Hymenopteran clades, the pruned genus-level tree includes 275 ingroup ant species from 268 described ant genera (82.0% of all extant ant genera), an increase of 105 genera compared to the most recent family-wide phylogenetic study (Moreau and Bell 2013). We will subsequently refer to the ingroup and outgroup taxa as "genera," for a total of 291 genera. Using these 291 genera, we then inferred another tree in RAxML using the same settings described above, and this genus-tree was used as a starting tree for divergence time estimation.

DIVERGENCE DATING

Divergence time estimation using Bayesian inference was conducted in BEAST v1.7 (Drummond et al. 2012) on the CIPRES computing cluster. The inferred maximum likelihood tree (291 genera) was used as the starting tree in BEAST. A total of 73 fossils, including 69 ingroup fossil taxa, were used to calibrate node age priors under a lognormal prior distribution with an offset corresponding to the minimum fossil ages (Table S3). A GTR + Γ model of sequence evolution was used for our gene-partitioned dataset, with parameters across partitions unlinked. All settings were implemented using BEAUti v1.7 (Drummond et al. 2012), which produces the XML file formatted for BEAST. After initial test runs, the MCMC length was set to 200 million generations (sampled every 10,000 generations), the upper bound of the ucld.mean parameter to 0.1, and the mean of ucld.stdev to 0.6, with all other settings left at the default. We then used Tracer v1.6 (Rambaut et al. 2014) with a burn-in of 10% to assess effective sample sizes for each parameter and ensure adequate convergence, and Tree Annotator v1.8.2 (Drummond et al. 2012) with 10% burn-in and a posterior probability limit of 50% to generate the dated maximum clade credibility tree. This MCC tree

was deposited in TreeBASE (URL: http://www.purl.org/phylo/treebase/phylows/study/TB2:S19313; http://www.treebase.org).

MORPHOLOGICAL AND ECOLOGICAL DATA

Trait data were compiled from information in the primary literature and online databases (Table S4; database deposited in the Dryad Digital Repository, URL: http://dx.doi.org/10.5061/dryad.st6sc). We collected data for five traits with a purported defensive function: cuticular spines ("spinescence" or "spines"), large eye size, worker polymorphism, unmodified venom-delivering sting ("sting"), and large colony size. Although each of these traits may have additional functions (e.g., foraging efficiency), they likely also serve a defensive role in many or most species in which they occur. Spines and the sting are both generally considered a direct defense against vertebrates or invertebrates (Lach et al. 2010; Schmidt 2014). Large eyes likely confer increased visual acuity allowing individuals to better avoid attackers (Cronin et al. 2014). Worker polymorphism, and in particular the recruitment of a soldier caste, is often used as a defense against arthropods (Lamon and Topoff 1981; Hunt 1983; Powell 2008; Kamhi et al. 2015). Large colony sizes may buffer against individual worker loss (Kaspari and Vargo 1995) or allow a colony to better defend itself against attackers (Adams 1990). In addition to these five traits, we collected data on three ecological niche traits: diet (herbivore, omnivore, or predator), nesting location (on the ground or arboreally), and foraging location (on the ground or arboreally). Historically, morphological and ecological data collection for ants has been heavily biased toward the worker caste, so we restricted our database to genera for which the worker caste is known. Traits were scored for each ant genus, and a genus was considered polymorphic for a trait if at least 10% of the species in the genus had an alternative trait state. In some cases, there was no information on a trait for a genus in the primary literature or online database records, but we assigned a state for the trait based on the likelihood of the trait state given background expectations. For example, a genus was considered to be monomorphic in the absence of documented evidence of polymorphism. These cases are clearly denoted in Table S4. As several analyses used in this study require traits to be categorical or binary, trait states were binned into discrete categories, and in some cases alternative binning schemes were used to test different hypotheses (see Table S5 for a discussion of trait state binning). For all analyses, polymorphic scores for a trait in a given genus were converted to the nonzero number (e.g., a score of "0&1" was converted to "1").

ANCESTRAL STATE RECONSTRUCTION

Ancestral state reconstructions were estimated for each trait at each node of the maximum clade credibility tree using the make.simmap and describe.simmap functions in phytools

v0.5-54 (Revell 2012), which utilize a stochastic character mapping approach (Huelsenbeck et al. 2003; Bollback 2006). The model of trait evolution used for each trait was determined by comparing the log likelihoods of three possible models: "Equal rates" (ER), "Symmetrical rates" (SYM), and "All rates different" (ARD), referring to rates of trait state changes for each trait. The model with the highest log-likelihood was chosen if the difference from each simpler model was greater than 2, which is a standard cut-off for this method (Paradis et al. 2004). We set the optimization method to "optim," and all other settings were left at the default. For each trait, we then ran 500 simulations, and the percentage of simulations for which each trait state was inferred at each node was then plotted as a pie chart on our maximum clade credibility tree. We also assessed the evolutionary lability of each trait using this approach, which returns the inferred number and type of changes occurring across the tree for each trait.

SHIFTS IN DIVERSIFICATION RATES

We used MEDUSA (Alfaro et al. 2009) to identify diversification rate shifts across the maximum clade credibility tree. MEDUSA uses a stepwise Akaike information criterion (AIC) method as implemented in geiger v2 (Harmon et al. 2008; Pennell et al. 2014). This method first assigns a background rate of diversification to an entire tree and calculates the likelihood for this singe rate regime under a birth-death (BD) or pure-birth (Yule) model. The program then searches for the most likely location for a rate shift based on AIC score. This more parameter-rich model is then compared to the model with only the background rate and is accepted if it results in a decrease in AIC score above a user-defined threshold (default = 4 AIC units). This process continues for models with an increasing number of rate shifts until there is no longer a decrease in AIC score greater than the user-defined threshold. To account for incomplete sampling, the number of species represented by each tip (i.e., genus in our case) was included (Bolton 2014; Table S4). We allowed the program to determine the best-fitting model of evolution for each shift (BD or Yule), and all other settings were left at the default. We then used Fisher's exact test (two-tailed) to investigate associations between rate shifts (increases and decreases) and defensive traits based on node states inferred from the ancestral state reconstruction. Preliminary analyses suggested that trait shifts in trait states very rarely occurred at the same nodes as rate shifts, so we only investigated node trait states, given that shifts in diversification due to a trait can also occur on a phylogeny downstream of the trait's origin (e.g., McKenna et al. 2009). However, although the rate shifts are distributed throughout our phylogeny, this method may suffer from pseudoreplication. We therefore consider this test to be a supplement to our core trait-based diversification analysis (below).

SISTER CLADE ANALYSIS

Assessing the influence of traits on diversification across clades is a classically difficult problem (Jablonski 2005; Vamosi and Vamosi 2011; Ng and Smith 2014; Maddison and FitzJohn 2015). New methods involving "state-dependent speciation and extinction" (SSE) models, including the BiSSE model and several derivatives, appeared to offer a promising solution to known issues with earlier methods (Ng and Smith 2014), such as the difficulty of inferring ancestral states for traits that also drive diversification (Maddison 2006). However, recent analyses have shown that the SSE suite of models frequently suffer from high rates of model inadequacy, sometimes resulting in the null model being rejected in 100% of tested cases, and thus often fail to properly account for phylogenetic relationships between species and clades (Maddison and FitzJohn 2015; Rabosky and Goldberg 2015). Preliminary analyses using our data and maximum clade credibility tree suggested that our tree is prone to the sort of model inadequacy highlighted by Rabosky and Goldberg (2015); therefore, we decided to instead use sister clade analysis. This more conservative method, while known to have somewhat low statistical power (Ng and Smith 2014), also explicitly ensures phylogenetic independence of included comparisons and is thus less prone to the artificially high rates of null model rejection seen in the SSE models (Maddison and FitzJohn 2015; Rabosky, pers. comm.). The results from our ancestral state reconstructions suggested that there were enough sister clades with differing states for all the traits included in this study to justify using sister clade analysis. Furthermore, most traits show numerous state transitions throughout the phylogeny, likely minimizing issues related to large imbalances in trait states in trait-based diversification analyses (Maddison 2006). Thus, we use sister clade analysis as a fairly conservative estimate of associations between traits and clade diversification.

We conducted sister clade comparisons based on plots of ancestral state reconstructions for our five morphological and three ecological traits. For each trait, sister clades were compared if the two clades differed in trait states and every genus in each clade shared a state for the trait. There were at least 10 and typically 20 or more such sister clades for our traits. We then implemented a model-based sister clade analysis that incorporates species numbers and time since divergence for each sister clade (Paradis 2011). This analysis uses a likelihood ratio test to compare the fit of two models of diversification––a null model and an alternative model where a trait increases or decrease rates of diversification. This method has been shown to be more powerful than previous techniques, and is implemented using the function richness.yule.test in the R package ape (Paradis et al. 2004). For each sister clade, we used the species numbers for each genus in our dataset (Table S4) and the dates of divergence inferred from our maximum clade credibility tree.
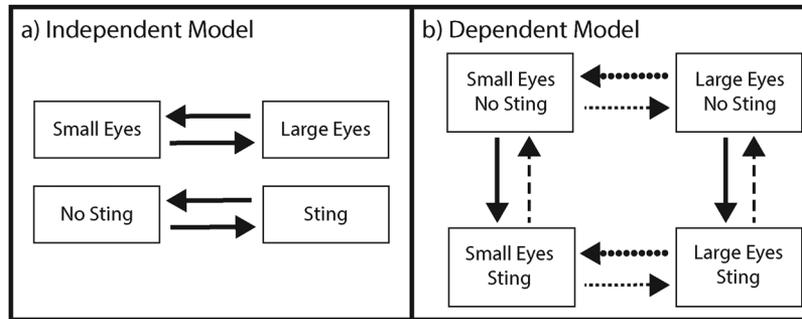
Figure 1. Example of models compared in corHMM to test for correlations between binary traits. The log-likelihood of the four-parameter, independent model (A) was compared to the eight-parameter, dependent model (B). If the eight-parameter model was significantly more likely (i.e., traits are correlated), an additional test (parameter restriction test) was conducted to determine the transition rate differences that significantly increase model likelihood. Four parameter restriction tests were conducted, with one pair of traits constrained to be equal in each test (B, indicated by line type). The likelihood of each of these seven-parameter models was compared to the full eight-parameter model, and parameter values are considered to be significantly different if there is a significant decrease in likelihood when the parameters are constrained.

ANALYSIS OF TRAIT CORRELATION

To assess the evidence for an evolutionary trade-off between defensive traits, we performed a phylogenetic maximum likelihood correlation test based on Pagel's method (Pagel 1994; Pagel and Meade 2013; Beaulieu and Oliver 2015). The phylogenetic correlation test was conducted using corDISC in corHMM v1.16 (Beaulieu et al. 2015), which tests hypotheses of correlated evolution between two binary traits. This test compares the log-likelihood of a four-parameter model, in which each trait evolves independently of the other trait, to the log-likelihood of an eight-parameter model that incorporates different transitions rates for one trait based on the state of a second trait (Fig. 1). The dependent (i.e., correlated) model is favored if the log-likelihood of the dependent model is significantly higher than that of the independent (i.e., uncorrelated) model, as determined by a likelihood ratio test (LRT) with four degrees of freedom.

In cases where the dependent model was favored, we tested additional hypotheses to determine which specific transition rates were affected by the associated trait. These hypotheses were tested by constraining certain pairs of parameters to be equal and comparing the constrained model to the full eight-parameter model (henceforth called the "parameter restriction test"). For example, to test for evidence of a trade-off between eye size and a sting, the transition rate from small eyes to large eyes in the absence of a sting was constrained to be equal to the transition rate from small eyes to large eyes in the presence of a sting (Fig. 1B). If the log-likelihood of this constrained, seven-parameter model is significantly lower than the full eight-parameter model (ascertained using a LRT with one degree of freedom), and the transition rate from small eyes to large eyes is higher in the absence of a sting, then there is support for a negative correlation (trade-off) between eye size and a sting. But if the rate from small eyes to large eyes is higher in the presence of a sting, then there is support for a positive correlation between the two traits. Thus, when there was evidence that two traits were correlated, the following were tested using the parameter restriction test (Fig. 1B):

1. Difference in transition rate from absence to presence of Trait 1 based on state of Trait 2.
2. Difference in transition rate from absence to presence of Trait 2 based on state of Trait 1.
3. Difference in transition rate from presence to absence of Trait 1 based on state of Trait 2.
4. Difference in transition rate from presence to absence of Trait 2 based on state of Trait 1.

These tests of correlation were conducted using the maximum clade credibility (MCC) tree for all possible pairwise comparisons between all traits, including for each different binning scheme for the ecological traits (diet, nesting, and foraging; see Table S5 for binning schemes).

Results
PHYLOGENETIC INFERENCE

The topology of the full species ML tree (666 tips, Fig. S1) and the maximum clade credibility (MCC) Bayesian genus-tree (291 tips, Fig. S2) are similar, and both are largely consistent with previous studies (LaPolla et al. 2010; Ward et al. 2010; Moreau and Bell 2013; Schmidt 2013; Brady et al. 2014; Ward et al. 2015). The posterior support values for most nodes in our tree are modest to strong (0.75–1.0), although a portion of the Ponerinae clade includes a relatively high number of nodes with low support (Fig. S2). The present tree differs in the relationships between subfamilies from the most recent family-wide phylogenetic study

(Moreau and Bell 2013) only in that Martialinae is inferred here to be the sister group to all other extant ants (with low support, PP = 0.41) as found in Rabeling et al. (2008) and Schmidt (2013), whereas Moreau et al. (2006), Kück et al. (2011), and Moreau and Bell (2013) found Leptanillinae to be sister. All other subfamily relationships match Moreau and Bell (2013). Major phylogenetic relationships at the tribe level are also consistent with previous subfamily analyses (Ward et al. 2010; Schmidt 2013; Ward et al. 2015), with a couple exceptions in Dorylinae (Brady et al. 2014). In this study, the Doryline genera *Amyrmex* and *Leptanilloides* were found within the "army ant" clade, whereas in Brady et al. (2014) these genera form their own clade. Furthermore, Brady et al. (2014) found *Vicinopone* as the basal-most Doryline genus, while in this study *Tanipone* takes this position.

DIVERGENCE DATING

The divergence-dated chronogram, generated from the BEAST analysis using the ML topology enforced as the starting tree, recovered a mean root age of 176.1 Mya [95% highest posterior density (HPD) 147.3 to 214.4 Mya] for crown-group ants (Fig. S2, Table S6). This tree includes 275 in-group genera, with 82% of extant ant genera represented. The dating of most major clades, including the largest subfamilies Myrmicinae, Formicinae, Dolichoderinae, and Ponerinae, is congruent with previous studies (Table S6). A notable exception to this is the inferred age of crown-group ants, which in this study is inferred to be about 25 million years earlier than the most recent family-wide analysis (Moreau and Bell 2013; Table S6). Although the inferred age of crown Formicidae and two subfamilies in our MCC tree are somewhat older than most recent studies, the majority of internal nodes are consistent with at least one of these studies, so we accepted this tree as adequate for subsequent analyses. Our MCC tree includes four genera without a known worker caste, which we excluded using the drop.tip function in ape (Paradis et al. 2004), resulting in a tree used for all downstream analyses that includes 271 ant genera (Fig. 2).

MORPHOLOGICAL AND ECOLOGICAL DATA

A dataset for eight scored morphological and ecological traits was generated for all 313 genera for which the worker caste is known, out of the total 326 extant ant genera (Table S4). Some data is missing for traits in some genera, but coverage is high across the 313 genera. Traits with missing data are sting (95.8% total coverage), diet (83.7%), nesting location (98.4%), foraging location (97.4%), and colony size (69.3%). Among the 271 ant genera in our tree, coverage is even higher for traits with missing data, which include sting (96.7%), diet (86.7%), foraging location (99.6%), and colony size (76.8%). For subsequent analyses involving a trait with incomplete taxonomic coverage, genera with missing data for that trait were pruned from the phylogeny using the drop.tip
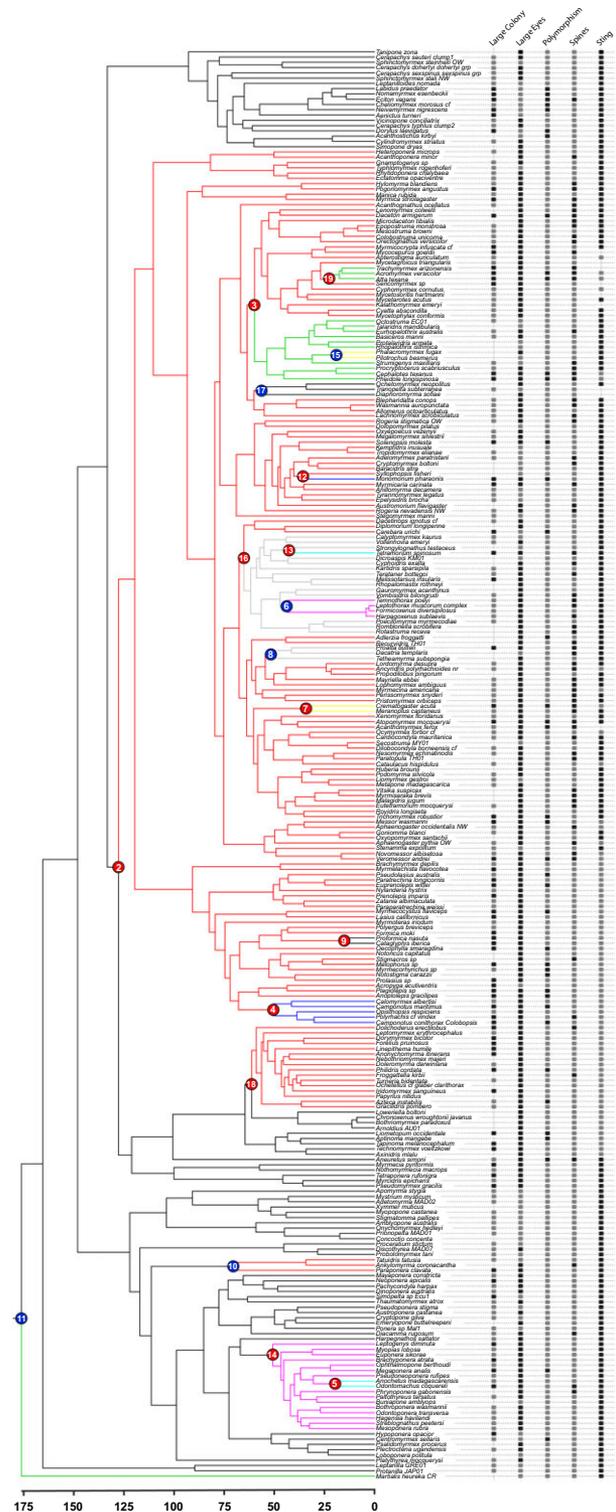
Figure 2. Dated maximum clade credibility (MCC) tree with trait states and MEDUSA rate shifts mapped. Outgroups are excluded. Scale bar is in millions of years before present. Trait states for each tip are denoted using a black square (trait present), a gray square (trait absent), or no square (no data). Nodes at which rate shifts occur are denoted by colored branches and labeled with a circle (red = rate increase, blue = rate decrease). The background rate begins at the root node.

Table 1. Rate shifts and rate shift parameters inferred using MEDUSA (+) indicates a significant rate increase, and (−) indicates a significant rate decrease. r = net diversification rate ($\lambda - \mu$, where λ = speciation rate, and μ = extinction rate), and ε = relative extinction rate ($\varepsilon = \mu/\lambda$).

Clades in rate regime	Direction [r]	# in Figure 2	r	ε
	Background	N/A	0.0309825	0.91215
Heteroponerinae, Ectatomminae, and large portion of Formicinae and Myrmicinae	+	2	0.0598134	0.74505
Octostruma, *Talaridris*, *Eurhopalothrix*, *Basiceros*, *Protalaridris*, *Rhopalothrix*, *Strumigenys*, *Procryptocerus*, *Cephalotes*, and *Pheidole*	+	3	0.1031564	0.88741
Calomyrmex, *Camponotus*, *Camponotus (Colobopsis)*, *Opisthopsis* and *Polyrhachis*	+	4	0.0606868	0.9801
Anochetus and *Odontomachus*	+	5	0.2278795	NA
Crematogaster and *Meranoplus*	+	7	0.1675758	NA
Proformica and *Cataglyphis*	+	9	0.2755019	NA
Monomorium	+	12	0.165836	NA
Tetramorium	+	13	0.1466766	NA
Leptogenys, *Myopias*, *Euponera*, *Euponera*, *Brachyponera*, *Ophthalmopone*, *Megaponera*, *Pseudoneoponera*, *Phrynoponera*, *Paltothyreus*, *Buniapone*, *Bothroponera*, *Odontoponera*, *Hagensia*, *Streblognathus*, and *Mesoponera*	+	14	0.0943411	NA
Calyptomyrmex, *Vollenhovia*, *Strongylognathus*, *Dicroaspis*, *Cyphoidris*, *Kartidris*, *Terataner*, *Melissotarsus*, *Rhopalomastix*, *Gauromyrmex*, *Vombisidris*, *Poecilomyrma*, *Romblonella*, and *Rotastruma*	+	16	0.068061	NA
Dolichoderus, *Leptomyrmex*, *Dorymyrmex*, *Forelius*, *Linepithema*, *Anonychomyrma*, *Nebothriomyrmex*, *Deleromyrma*, *Philidris*, *Froggattella*, *Turneria*, *Ochetellus*, *Iridomyrmex*, *Papyrius*, *Azteca*, and *Gracilidris*	+	18	0.0833777	NA
Trachymyrmex, *Acromyrmex*, and *Atta*	+	19	0.2129122	NA
Temnothorax, *Leptothorax*, *Formicoxenus*, and *Harpagoxenus*	−	6	0.0018187	0.9996
Proatta, *Dacatria*, and *Tetheamyrma*	−	8	0.0069088	NA
Tatuidris, *Ankylomyrma*, and *Paraponera*	−	10	0.0057103	NA
Martialis	−	11	0	NA
Phalacromyrmex and *Pilotrochus*	−	15	0	NA
Ochetomyrmex, *Tranopelta*, and *Diaphoromyrma*	−	17	0.0169692	NA

function in ape (Paradis et al. 2004), unless the analysis could incorporate missing data (e.g., correlation test in corHMM).

ANCESTRAL STATE RECONSTRUCTION

Ancestral state reconstructions revealed a range of evolutionary lability across the eight morphological and ecological traits (Figs. S3–S10). The least labile trait was a functional sting, while the most labile traits included nesting location and colony size (Table S7). Despite the general lability of colony size across all ants, the evolution of large colony size only occurred once each in several subfamilies (Fig. S3), as seen in the recent analysis

of Burchill and Moreau (2016). Among the 14 changes in sting presence across the tree, none were inferred as secondary gains. This directionality of trait state transitions was also found, to a lesser degree, for spines (loss of spines more than twice as likely as gain), and eye size (eye size increase 50% more common than decrease). Furthermore, the majority of shifts in diet were to a predacious or herbivorous diet, with only 14 shifts to omnivory.

SHIFTS IN DIVERSIFICATION RATE

The MEDUSA analysis of our dated phylogeny detected 18 significant rate shifts in diversification, including 12 rate increases

Table 2. Associations between rate shifts and defensive trait states at nodes with rate shifts inferred by MEDUSA. The percentage of rate decreases with a trait present was compared to the percentage of rate increases with the trait present.

Trait	% state 1 at rate decrease	% state 1 at rate increase	Fisher's exact test (P-value)
Colony size	0%	33%	0.2605
Eye size	50%	92%	0.0833
Polymorphism	0%	8%	1
Spines	50%	42%	1
Sting	100%	67%	0.2451
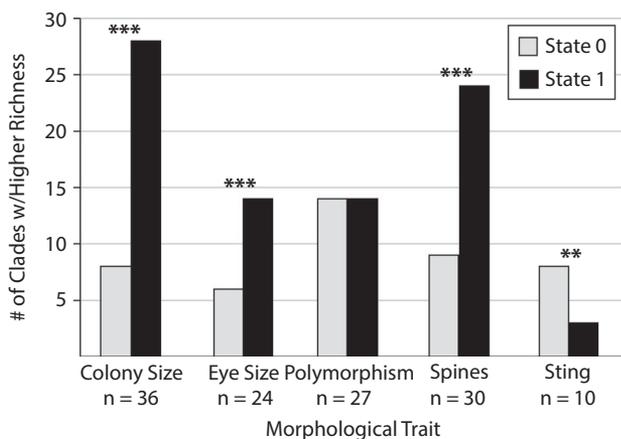
Figure 3. Results of sister clade analysis of morphological traits and diversification. An association between diversification and trait states (0 or 1) was assessed using a model-based likelihood ratio test (Paradis 2011; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

and six rate decreases (Table 1). The largest single shift was a rate increase that includes four subfamilies: Heteroponerinae, Ectatomminae, Myrmicinae, and Formicinae (Table 1, Fig. 2). There were no rate shifts detected in Dorylinae, Pseudomyrmecinae, Amblyoponinae, Aneuretinae, or Proceratiinae (Fig. 2).

Based on Fisher's exact test and the ancestral state reconstructions at nodes where rate shifts occurred (Table S8), there is a trend of marginal significance suggesting that large eye size is associated with rate increases ($P = 0.08$, Table 2), as well as a positive but nonsignificant association between large colony size and rate increases ($P = 0.26$, Table 2). There was also a nonsignificant positive association between a sting and rate decreases ($P = 0.25$, Table 2). Spines and polymorphism were not associated with rate shifts.

SISTER CLADE ANALYSIS

The sister clade analyses provided support for the impact of several traits on diversification. Among morphological defensive traits, colony size, eye size, and spines were associated with increased diversification rate ($P < 0.001$ for all traits; Fig. 3) and

the sting was associated with decreased diversification rate ($P = 0.007$, Fig. 3), while polymorphism was not associated with diversification. Among ecological traits, broad foraging, and broad nesting were both associated with increased diversification rate ($P < 0.001$ and $P = 0.002$, respectively), while ground foraging and nesting and arboreal foraging and nesting were all associated with decreased diversification rate ($P < 0.001$, $P = 0.016$, $P = 0.035$, and $P < 0.001$, respectively; Fig. S11). Diet was not associated with diversification.
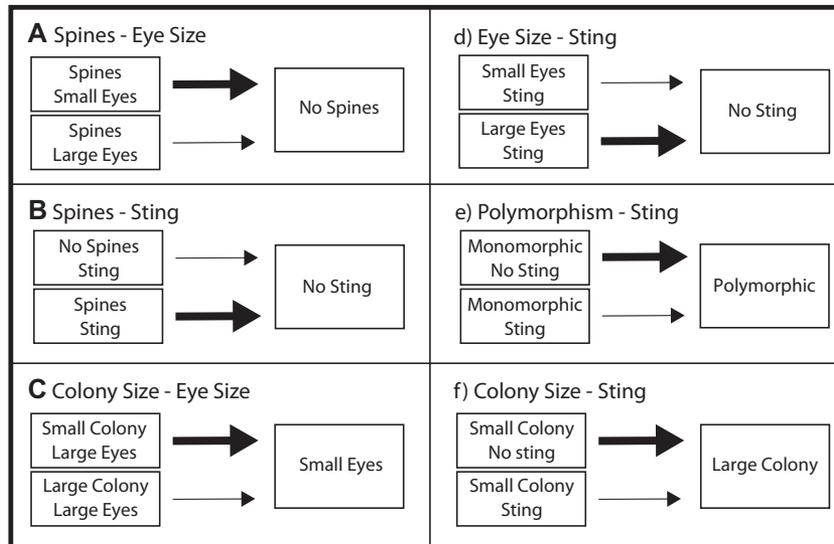
TRAIT CORRELATION

Among the 55 phylogenetically informed pairwise tests for correlation, the model that included correlated transition rates was favored for 30 pairs of traits (Table S9). These included seven morphological trait pairs and 23 morphological-ecological trait pairs. Among these 30 correlated trait pairs, the parameter restriction test found that the model with a difference in a tested transition rate was significantly favored for 18 trait pairs (Table S9, Fig. 4). Among morphological traits pairs, the transition rates in the favored models supported a positive association between large eye size and large colony size as well as between large eye size and spines. The transition rates in the favored models also supported negative associations between the sting and all four of the other traits (large colony size, large eye size, polymorphism, and spines).

Among the 23 correlated morphological-ecological trait pairs, the parameter restriction test found that the model with a difference in a tested transition rate was significantly favored for 12 pairs (Table S9). Eye size was positively associated with omnivory, and negatively associated with a predacious diet and ground nesting. Polymorphism was both positively and negatively associated with an herbivorous diet, and negatively associated with a predacious diet. Spines were positively associated with an herbivorous diet, and both positively and negatively associated with broad foraging. A sting was positively associated with a predacious diet but negatively associated with arboreal foraging and an herbivorous diet. Colony size was positively associated with an herbivorous diet, and negatively associated with a predacious diet.

Discussion

Species diversification and morphological evolution are shaped by diverse processes, including niche adaptation (Evans et al. 2009; Ricklefs 2010), developmental constraints (Porto et al. 2015), key innovations (Rainford et al. 2014; Weber and Agrawal 2014), interspecific competition (Rabosky 2013; Price et al. 2014), and predation (Langerhans et al. 2004; Arbuckle and Speed 2015). Here, we assessed the impact of evolutionary trade-offs among defensive traits on the evolution of the ants, an ecologically

Figure 4. Results of the parameter restriction tests among significantly correlated morphological trait pairs. The 8-parameter model was compared to 7-parameter models in which one pair of parameters are constrained to be equal (see Methods). In (A)–(F), among all parameter pairs investigated, the unconstrained parameter difference that significantly increased model likelihood is shown. A thicker arrow indicates the transition parameter that is larger than its corresponding paired parameter in the model favored by the test.

dominant clade of insects exhibiting remarkable morphological diversity (Hölldobler and Wilson 1990). We inferred the most complete phylogeny of ants to date, covering 82% of all currently recognized ant genera, and produced the first family-wide database of morphological and ecological traits that includes every described genus with a known worker caste (Table S4). Using this phylogeny and database of traits, we implemented a number of analyses to determine patterns of diversification across ants, assess the evolutionary lability of multiple ecological and morphological traits as well as their association with diversification, and detect correlations between these traits to test the hypothesis that the evolution of defensive traits is constrained by a trade-off.

TRAIT EVOLUTION IN ANTS

Three of our five morphological defensive traits exhibited significant directionality in losses and gains. In particular, eye size increases were 50% more common than decreases, spine loss was more than two times as common as spine gain, and the loss of a sting occurred repeatedly while no gains of a sting were inferred (Table S7). Furthermore, evolutionary lability varied considerably––eye size, polymorphism, and spines all changed more than four times as often as the sting, which was highly stable. The most labile trait is colony size, with 238 inferred changes across the 271 genera (Table S7). However, despite the large number of changes in colony size across the tree, large colony size in the subfamilies Dorylinae, Dolichoderinae, Formicinae, and Pseudomyrmecinae arose only once in each subfamily with subsequent losses in each clade, based on node reconstructions (Fig. S3). This pattern of variation in colony size evolution is

particularly interesting, as colony size has been shown to be the ecological equivalent of body size in ants (Kaspari and Vargo 1995; Hou et al. 2010; Dornhaus et al. 2012). In fact, a recent study found that macroevolutionary trends in colony size partially follow the predictions of a hypothesis initially developed to explain trends in body size evolution, where large colony sizes are generally maintained and size changes occur over relatively incremental steps rather than larger jumps (Burchill and Moreau 2016). Body size is linked to diversification rate in many taxa (Maurer et al. 1992; Wollenberg et al. 2011; Rabosky et al. 2013), and is a key feature of several ecological and evolutionary theories such as Bergmann's rule (Meiri and Dayan 2003), which has been observed for colony size in ants (Kaspari and Vargo 1995). Given the general pattern of repeated evolution of large colony size across our phylogeny as well as recent evidence of the importance of colony size trends in ant evolution, investigations of colony size evolution on a broad scale across ants, including comparisons between colony size versus body size, is likely to be a promising avenue for future research.

We also detected differences in evolutionary lability among the three ecological traits of diet (herbivorous, predacious, or omnivorous), nesting (arboreal and/or ground), and foraging (arboreal and/or ground). Diet appears to be more stable than spatial ecological niche, with a trend toward specialization (predation or herbivory), whereas spatial ecological niche (nesting and foraging locations) changes much more often, but with no significant trends in directionality. The trend toward diet specialization is consistent with a previous study on diet transitions in phytophagous insects, which found that the transition rate from diet generalization

to specialization was greater than the reverse transition rate (Nosil 2002). Furthermore, our finding of frequent evolutionary changes in nesting location is consistent with a recent study of habitat changes across all ants (Lucky et al. 2013). It is possible that specializing in diet minimizes interspecific competition, while frequent changes in spatial niche, including many niche expansions, decrease the likelihood of extinction due to stochastic environmental change (Futuyma and Moreno 1988; Jablonski 2008).

DEFENSIVE TRAITS AND DIVERSIFICATION

We find support for an association between some defensive traits and shifts in rates of diversification. In particular, large eye size and large colony size were associated with rate increases while the presence of a sting was associated with rate decreases. Although the statistical support for these trends was modest to weak, our sister clade analysis found a significant association between both large eye size and large colony size and elevated rates of diversification as well as a significant association between a sting and decreased rates of diversification (Fig. 3), consistent with the trends from the rate shift analysis. The sister clade analysis also found elevated diversification significantly associated with spinescence, but did not find a significant association between polymorphism and diversification (Fig. 3).

There are several mechanisms that could cause spines, large eye size, and large colony sizes to increase diversification rate. One explanation for why certain defensive traits might drive diversification is the escape-and-radiate hypothesis, which states that species that evolve traits allowing them to escape from natural predators may be more able to evolve into novel niches and subsequently radiate (Schluter 2000; Arbuckle and Speed 2015). The predictions of this hypothesis suggest that both spines and the increased visual acuity afforded by large eye size allow species to avoid predation, while large colony size diminishes the cost of individuals lost to predation, driving increased diversification. In fact, it is notable that the two ecological traits associated with elevated diversification represent ecological niche expansion, namely broad foraging and broad nesting, while some niche specialization (ground foraging and nesting) is associated with lower rates of diversification (Fig. S11). This expansion of ecological niche can promote diversification through ecological opportunity (Moreau et al. 2006; Yoder et al. 2010; Price et al. 2014) consistent with predictions of the escape-and-radiate hypothesis. Our results are also consistent with recent evidence that herbivory is not associated with elevated diversification in Hymenoptera despite an association in many other insect orders (Wiens et al. 2015). We suggest that diversification in ants is promoted by morphological traits that buffer species against enemy attack and an expansion in ecological niche traits that may promote speciation and/or slow extinction. However, further work on ecologically relevant morphological traits is needed to elucidate the mechanistic forces driving trait-based diversification in ants.

EVOLUTIONARY TRADE-OFFS IN DEFENSIVE TRAITS

The results of our corHMM analyses support the existence of a trade-off between investing in a sting versus a suite of traits including polymorphism, large eye size, large colony size, and spines (Fig. 4). In particular, the loss of a sting is more likely when spines or large eyes are present, and the gain of polymorphism or large colony size is more likely when a sting is absent. Furthermore, the loss of spines is more likely when eyes are small, and the loss of large eyes is more common when colony size is small. These data therefore support an evolutionary trade-off between a sting and the other defensive traits tested, and also suggest that spines, large colony size, and large eye size may form a defensive suite.

This finding of a suite of traits that trade off with a sting is consistent with recent studies in plants that suggest that defense is characterized more by defense "syndromes" of correlated traits rather than trade-offs between individual traits (Agrawal 2007, 2011). These traits that trade off with a sting may therefore be interdependent. For example, an increase in colony size may allow for the division of labor through polymorphism, which would otherwise be too costly for species with smaller colonies (Tschinkel 1988; Thomas and Elgar 2003). Similarly, large eyes may be more beneficial when colony sizes are large due to a heightened risk of exposure and subsequent predation. Furthermore, there may be a trade-off between a "strength-in-numbers" approach (e.g., large colony size) and individual worker trait investment (e.g., sting) for active defense strategies.

Considered in conjunction with our trait-based diversification results, the trade-offs that we find here have interesting macroevolutionary implications. Spines, large colony size, and large eyes are all associated with elevated rates of diversification and trade off with a sting, which is associated with decreased rates of diversification. Thus, selection to evolve a sting may be beneficial in the short term but ultimately suppress rates of diversification over evolutionary time, as having a sting constrains a given clade from evolving other traits that are drivers of diversification. This constraint may be driven by the costly production of a sting and its associated chemicals, as well as the energy cost resulting from actively stinging a given attacker, and these additional costs may not be fully compensated for by the alternative use of a sting in prey capture. Therefore, our data suggest that sting production at the expense of other defensive traits may be a type of "evolutionary dead end" in ants (Agnarsson et al. 2006), and that dynamics at the species or population level (e.g., selection for a sting) may generate unexpected evolutionary dynamics at broader taxonomic levels (e.g., lower rates of diversification in one clade relative to other clades) (Jablonski 2008).

Conclusion

We have presented here the first multitrait investigation of morphological evolution across all ants, using a well-sampled molecular phylogeny to address the role of trait trade-offs in ant evolution. Our trait database is the first to include information on every described extant genus with a known worker caste, and we hope that making the database public and editable will serve to enhance further research. We find strong evidence for an evolutionary trade-off between sting production and a suite of defensive traits including spines, large colony size, and large eyes, supported by both phylogenetic and nonphylogenetic analyses. Our results suggest that defensive traits significantly influence macroevolutionary patterns in ants. Given that there is little data on the particular ecological roles of these traits for the majority of ants (Lach et al. 2010), we emphasize the need for more work on the functional ecology of defensive traits in this highly diverse and ecologically important group of insects. Such work is crucial to expand our understanding of ant evolution, from ecological process to evolutionary outcome.

DATA ARCHIVING

The doi for our data is 10.5061/dryad.st6sc.

LITERATURE CITED

Adams, E. S. 1990. Boundary disputes in the territorial ant *Azteca trigona*: effects of asymmetries in colony size. Anim. Behav. 39:321–328.

Agnarsson, I., L. Avilés, J. A. Coddington, and W. P. Maddison. 2006. Sociality in theridiid spiders: repeated origins of an evolutionary dead end. Evolution 60:2342–2351.

Agrawal, A. A. 2007. Macroevolution of defense strategies. Trends Ecol. Evol 22:103–109.

Agrawal, A. A. 2011. Current trends in the evolutionary ecology of plant defence. Funct. Ecol. 25:420–432.

Alfaro, M. E., F. Santini, C. Brock, H. Alamillo, A. Dornburg, D. L. Rabosky, G. Carnevale, and L. J. Harmon. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc. Nat. Acad. Sci. 106:13410–13414.

Arbuckle, K., and M. P. Speed. 2015. Antipredator defenses predict diversification rates. Proc. Nat. Acad. Sci. 112:13597–13602.

Arnold, S. J. 1992. Constraints on phenotypic evolution. Am. Nat. 140:S85–S107.

Beaulieu, J. M., and J. C. Oliver. 2015. corHMM: hidden Markov models in R, version 1.16.

Bennett, A. F., and R. E. Lenski. 2007. An experimental test of evolutionary trade-offs during temperature adaptation. Proc. Nat. Acad. Sci. 104:8649–8654.

Blaimer, B. B., S. G. Brady, T. R. Schultz, M. W. Lloyd, B. L. Fisher, and P. S. Ward. 2015. Phylogenomic methods outperform traditional multilocus approaches in resolving deep evolutionary history: a case study of formicine ants. BMC Evol. Biol. 15:e271.

Blaimer, B. B., S. G. Brady, T. R. Schultz, and B. L. Fisher. 2015. Functional and phylogenetic approaches reveal the evolution of diversity in a hyper diverse biota. Ecography 38:901–912.

Bollback, J. P. 2006. SIMMAP: stochastic character mapping of discrete traits on phyogenies. BMC Bioinformatics 7:88.

Bolton, B. 2014. An online catalog of the ants of the world. Available at http://antcat.org. Accessed June 2015.

Brady, S. G., T. R. Schultz, B. L. Fisher, and P. S. Ward. 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. Proc. Nat. Acad. Sci. 103:18172–18177.

Brady, S. G., B. L. Fisher, T. R. Schultz, and P. S. Ward. 2014. The rise of army ants and their relatives: diversification of specialized predatory doryline ants. BMC Evol. Biol. 14:93.

Burchill, A. T., and C. S. Moreau. 2016. Colony size evolution in ants: macroevolutionary trends. Insectes Soc. 63:291–298.

Cronin, T. W., S. Johnsen, N. J. Marshall, and E. J. Warrant. 2014. Visual ecology. Princeton Univ. Press, Princeton, New Jersey.

Crother, B. I., M. E. White, and A. D. Johnson. 2007. Inferring developmental constraint and constraint release: primordial germ cell determination mechanisms as examples. J. Theor. Biol. 248:322–330.

Darwin, C. 1859. On the origin of species by means of natural selection. John Murray, London, UK.

DeWitt, T. J., B. W. Robinson, and D. S. Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. Evol. Ecol. Res. 2:129–148.

de Vos, M. G. J., A. Dawid, V. Sunderlikova, and S. J. Tans. 2015. Breaking evolutionary constrain with a tradeoff ratchet. Proc. Nat. Acad. Sci. 112:14906–14911.

Dornhaus, A., S. Powell, and S. Bengston. 2012. Group size and its effects on collective organization. Ann. Rev. Entomol. 57:123–141.

Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29:1969–1973.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

Endara, M., A. Weinhold, J. E. Cox, N. L. Wiggins, P. D. Coley, and T. A. Kursar. 2015. Divergent evolution in antiherbivore defences within species complexes at a single Amazonian site. J. Ecol. 103:1107–1118.

Evans, M. E. K., S. A. Smith, R. S. Flynn, and M. J. Donoghue. 2009. Climate, niche evolution, and diversification of the "Bird-Cage" Evening Primroses (*Oenothera*, Sections *Anogra* and *Kleinia*). Am. Nat. 173:225–240.

Ferenci, T. 2016. Trade-off mechanisms shaping the diversity of bacteria. Trends Microbiol. 24:209–223.

Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Ann. Rev. Ecol. Syst. 19:207–233.

Ge, D., D. Chester, J. Gómez-Zurita, L. Zhang, X. Yang, and A. P. Volger. 2011. Anti-predator defence drives parallel morphological evolution in flea beetles. Proc. R. Soc. Lond. B. 278:2133–2141.

Ghalambor, C. K., D. N. Reznick, and J. A. Walker. 2004. Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). Am. Nat. 164:38–50.

Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129–131.

Heinen-Kay, J. L., K. E. Morris, N. A. Ryan, S. L. Byerley, R. E. Venezia, M. N. Peterson, and R. B. Langerhans. 2015. A trade-off between natural and sexual selection underlies diversification of a sexual signal. Behav. Ecol. 26:533–542.

Herrel, A., J. Podos, B. Vanhooydonck, and A. P. Hendry. 2009. Force-velocity trade-off in Darwin's finch jaw function: a biomechanical basis for ecological speciation? Funct. Ecol. 23:119–125.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Harvard Univ. Press, Oxford, MA.

Holzman, R., D. C. Collar, S. A. Price, C. D. Hulsey, R. C. Thomson, and P. C. Wainwright. 2012. Biomechanical trade-offs bias rates of evolution in the feeding apparatus of fishes. Proc. R Soc. Lond. B 279:1287–1292.

Hou, C., M. Kaspari, H. B. Cander Zanden, and J. F. Gillooly. 2010. Energetic basis of colonial living in social insect. Proc. Nat. Acad. Sci. 107:3634–3638.

Huelsenbeck, J. P., R. Nielsen, and J. P. Bollback. 2003. Stochastic mapping of morphological characters. Syst. Biol. 52:131–158.

Hunt, J. H. 1983. Foraging and morphology in ants: the role of vertebrate predators as agents of natural selection. *In* P. Jaisson, ed. Social insects in the tropics. Presses de l'Université Paris XIII, Paris.

Jablonski, D. 2005. Mass extinctions and macroevolution. Paleobiology 31:192–210.

———. 2008. Species selection: theory and data. Ann. Rev. Ecol. Evol. Syst. 39:501–524.

Kamhi, J. F., K. Nunn, S. K. A. Robson, and J. F. A Traniello. 2015. Polymorphism and division of labour in a socially complex ant: neuromodulation of aggression in the Australian weaver ant, *Oecophylla smaragdina*. Proc. R Soc. Lond. B 282:20150704.

Kaspari, M., and E. L. Vargo. 1995. Colony size as a buffer against seasonality: Bergmann's Rule in social insects. Am. Nat. 145:610–632.

Krebs, J. R. 1978. Optimal foraging: decision rules for predators. *In* Krebs, J. R., and N. B. Davies, eds. Behavioral ecology: an evolutionary approach. Blackwell, Oxford, MA.

Kück, P., F. Hita Garcia, B. Misof, and K. Meusemann. 2011. Improved phylogenetic analyses corroborate a plausible position of *Martialis heureka* in the ant tree of life. PLOS ONE 6:e21031.

Lach, L., C. L. Parr, and K. L. Abott. 2010. Ant ecology. Oxford Univ. Press, Oxford.

Lamon, B., and H. Topoff. 1981. Avoiding predation by army ants: defensive behaviours of three ant species of the genus *Camponotus*. Anim. Behav. 29:1070–1081.

Langerhans, R. B., C. A. Layman, A. M. Shokrollahi, and T. J. DeWitt. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. Evolution 58:2305–2318.

Lapolla, J. S., S. G. Brady, and S. O. Shattuck. 2010. Phylogeny and taxonomy of the *Prenolepis* genus-group of ants (Hymenoptera: Formicidae). Syst. Entomol. 35:118–131.

Lucky, A., M. D. Trautwein, B. S. Guénard, M. D. Weiser, and R. R. Dunn. 2013. Tracing the rise of ants—out of the ground. PLOS ONE 8:e84012.

Maddison, W. P. 2006. Confounding asymmetries in evolutionary diversification and character change. Evolution 60:1743–1746.

Maddison, W. P., and D. R. Maddison. 2015. Mesquite: a modular system for evolutionary analysis. Version 3.02. Available at http://mesquite project.org.

Maddison, W. P., and R. G. FitzJohn. 2015. The unsolved challenge to phylogenetic correlation tests for categorical characters. Syst. Biol. 64:127–136.

Maurer, B. A., J. H. Brown, and R. D. Rusler. 1992. The micro and macro in body size evolution. Evolution 46:939–953.

McKenna, D. D., A. S. Sequeira, A. E. Marvaldi, and B. D. Farrell. 2009. Temporal lags and overlap in the diversification of weevils and flowering plants. Proc. Nat. Acad. Sci. 106:7083–7088.

Meiri, S., and T. Dayan. 2003. On the validity of Bergmann's rule. J. Biogeogr. 30:331–351.

Mikolajewski, D. J., M. De Block, J. Rolff, F. Johansson, A. P. Beckerman, and R. Stoks. 2010. Predator-driven trait diversification in a dragonfly genus: covariation in behavioral and morphological antipredator defense. Evolution 64:3327–3335.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 *In* Proceedings of the gateway computing environments workshop (GCE), New Orleans, LA. Available at: http://www.phylo.org/sub_sections/portal/cite.php.

Mooney, K. A., R. Halitschke, A. Kessler, and A. A. Agrawal. 2010. Evolutionary trade-offs in plants mediate the strength of trophic cascades. Science 327:1642–1644.

Moreau, C. S., C. D. Bell, R. Vila, S. B. Archibald, and N. E. Pierce. 2006. Phylogeny of the ants: diversification in the age of angiosperms. Science 312:101–104.

Moreau, C. S. 2008. Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). Mol. Phylogenet. Evol. 48:224–239.

Moreau, C. S., and C. D. Bell. 2013. Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. Evolution 67:2240–2257.

Muchhala, N. 2007. Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. Am. Nat. 169:494–504.

Ng, J., and S. D. Smith. 2014. How traits shape trees: new approaches for detecting character state-dependent lineage diversification. J. Evol. Biol. 27:2035–2045.

Nosil, P. 2002. Transition rates between specialization and generalization in phytophagous insects. Evolution 56:1701–1706.

Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc. R. Soc. Lond. B 255:37–45.

Pagel, M., and A. Meade. 2013. BayesTraits v. 2.0. Available at http://www.evolution.rdg.ac.uk/BayesTraits.html

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Paradis, E. 2011. Shift in diversification in sister-clade comparisons: a more powerful test. Evolution 66:288–295.

Pennell, M. W., J. M. Eastman, G. J. Slater, J. W. Brown, J. C. Uyeda, R. G. FitzJohn, M. E. Alfaro, and L. J. Harmon. 2014. geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. Bioinformatics 30:2216–2218.

Pie, M. R., and J. F. A. Traniello. 2007. Morphological evolution in a hyperdiverse clade: the ant genus *Pheidole*. J. Zool. 271:99–109.

Porto, A., H. Sebastião, S. E. Pavan, J. L. Vandeberg, G. Marroig, and J. M. Cheverud. 2015. Rate of evolutionary change in cranial morphology of the marsupial genus *Mondelphis* is constrained by the availability of additive genetic variation. J. Evol. Biol. 28:973–985.

Powell, S., and N. R. Franks. 2006. Ecology and the evolution of worker morphological diversity: a comparative analysis with *Eciton* army ants. Funct. Ecol. 20:1105–1114.

Powell, S. 2008. Ecological specialization and the evolution of a specialized caste in *Cephalotes* ants. Funct. Ecol. 22:902–911.

Price, S. L., S. Powell, D. J. C. Kronauer, L. A. P. Tran, N. E. Pierce, and R. J. Wayne. 2014. Renewed diversification is associated with new ecological opportunity in the Neotropical turtle ants. J. Evol. Biol. 27:252–258.

Price, S. L., R. E. Etienne, and S. Powell. 2016. Tightly congruent bursts of lineage and phenotypic diversification identified in a continental ant radiation. Evolution 70:903–912.

Price, T. D., D. M. Hooper, C. D. Buchanan, U. S. Johansson, D. T. Tietze, P. Alström, U. Olsson, M. Ghosh-Harihar, F. Ishtiaq, S. G. Gupta, et al. 2014. Niche filling slows the diversification of Himalayan songbirds. Nature 509:222–225.

Rabeling, C., J. M. Brown, and M. Verhaagh. 2008. Newly discovered sister lineage sheds light on early ant evolution. Proc. Nat. Acad. Sci. 105:14913–14917.

Rabosky, D. L. 2013. Diversity-dependence, ecological selection, and the role of competition in macroevolution. Ann. Rev. Ecol. Evol. Syst. 44:481–502.

Rabosky, D. L., and E. E. Goldberg. 2015. Model inadequacy and mistaken inferences of trait-dependent speciation. Syst. Biol. 64:340–355.

Rabosky, D. L., F. Santini, J. Eastman, S. A. Smith, B. Sidlauskas, J. Chang, M. E. Alfaro. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nat. Comm. 4:1958.

Rainford, J. L., M. Hofreiter, D. B. Nicholson, and P. J. Mayhew. 2014. Phylogenetic distribution of extant richness suggests metamorphosis is a key innovation driving diversification in insects. PLOS ONE 9:e109085.

Rambaut, A., M. A. Suchard, D. Xie, and A. J. Drummond. 2014. Tracer v1.6. Available at http://beast.bio.ed.ac.uk/Tracer.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R–project.org/.

Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3:217–223.

Ricklefs, R. E. 2010. Evolutionary diversification, coevolution between populations and their antagonists, and the filling of niche space. Proc. Nat. Acad. Sci. 107:1265–1272.

Sarnat, E. M., and C. S. Moreau. 2011. Biogeography and morphological evolution in a Pacific island ant radiation. Mol. Ecol. 20:114–130.

Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. Ecology 76:82–90.

———. 2000. The ecology of adaptive radiation. Oxford Univ. Press, Oxford.

Schmidt, C. 2013. Molecular phylogenetics of ponerine ants (Hymenoptera: Formicidae: Ponerinae). Zootaxa 3647:201–250.

———. 2014. Evolutionary responses of solitary and social Hymenoptera to predation by primates and overwhelmingly powerful vertebrate predators. J. Hum. Evol. 71:12–19.

Shoval, O., H. Sheftel, G. Shinar, Y. Hart, O. Ramote, A. Mayo, E. Dekel, K. Kavanagh, and U. Alon. 2012. Evolutionary trade-offs, Pareto optimality, and the geometry of phenotype space. Science 336:1157–1160.

Silva, R. R., and C. R. F. Brandão. 2014. Ecosystem-wide morphological structure of leaf-litter ant communities along a tropical latitudinal gradient. PLOS ONE 9:e93049.

Stamatakis, A. 2014. RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.

Stearns, S. C. 1989. Trade-offs in life-history evolution. Funct. Ecol. 3:259–268.

Svanbäck, R., and P. Eklöv. 2003. Morphology dependent foraging efficiency in perch: a trade-off for ecological specialization? Oikos 102:273–284.

Thomas, M. L., and M. A. Elgar. 2003. Colony size affects division of labour in the ponerine ant *Rhytidoponera metaliica*. Naturwissenschaften 90:88–92.

Tilman, D. 2011. Diversification, biotic interchange, and the Universal Trade-Off Hypothesis. Am. Nat. 178(3):355–371.

Tschinkel, W. R. 1988. Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. Behav. Ecol. Sociobiol. 22:103–115.

Vamosi, J. C., and S. M. Vamosi. 2011. Factors influencing diversification in angiosperms: at the crossroads of intrinsic and extrinsic traits. Am. J. Bot. 98:460–471.

Ward, P. S., S. G. Brady, B. L. Fisher, and T. R. Schultz. 2010. Phylogeny and biogeography of Dolichoderine ants: effects of data partitioning and relict taxa on historical inference. Syst. Biol. 59:342–362.

Ward, P. S., S. G. Brady, B. L. Fisher, and T. R. Schultz. 2015. The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). Syst. Entomol. 40:61–81.

Weiser, M. D., and M. Kaspari. 2006. Ecological morphospace of New World ants. Ecol. Entomol. 31:131–142.

Weber, M. G., and A. A. Agrawal. 2014. Defense mutualisms enhance plant diversification. Proc. Nat. Acad. Sci. 111:16442–16447.

Werner, E. E., and B. R. Anholt. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am. Nat. 142:242–272.

Wiens, J. J., R. T. Lapoint, and N. K. Whiteman. 2015. Herbivory increases diversification across insect clades. Nat. Comm. 6:8370.

Wilson, E. O. 1959. Some ecological characteristics of ants in New Guinea rain forests. Ecology 40:437–447.

Wollenberg, K. C., D. R. Vieites, F. Glaw, and M. Vences. 2011. Speciagtion in little: the role of range and body size in the diversification of Malagasy mantellid frogs. BMC Evol. Biol. 11:217.

Yoder, J. B., E. Clancey, S. Des Roches, J. M. Eastman, L. Gentry, W. Godsoe, T. J. Hagey, D. Jochimsen, B. P. Oswald, J. Robertson, et al. 2010. Ecological opportunity and the origin of adaptive radiations. J. Evol. Biol. 23:1581–1596.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Maximum likelihood tree inferred in RAxML, including 648 in-group species and 18 out-group species. Nodes are labeled with bootstrap support values and branch lengths are proportional to substitution/site as indicated by the bottom legend inset.

Figure S2. Dated Bayesian maximum clade credibility (MCC) tree, including 275 in-group and 16 out-group species. Nodes are labeled with posterior probability (PP) values. Scale bar is in millions of years before present.

Figure S3. Ancestral state reconstruction (stochastic character mapping) for colony size. The crown nodes for subfamilies noted in the main text are also labeled A-D.

Figure S4. Ancestral state reconstruction (stochastic character mapping) for eye size.

Figure S5. Ancestral state reconstruction (stochastic character mapping) for polymorphism.

Figure S6. Ancestral state reconstruction (stochastic character mapping) for spines.

Figure S7. Ancestral state reconstruction (stochastic character mapping) for sting.

Figure S8. Ancestral state reconstruction (stochastic character mapping) for diet.

Figure S9. Ancestral state reconstruction (stochastic character mapping) for foraging location.

Figure S10. Ancestral state reconstruction (stochastic character mapping) for nesting location.

Figure S11. Results of sister clade analyses of ecological traits and diversification. An association between diversification and trait states (0 or 1) is assessed using a model-based likelihood ratio test (Paradis 2011; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

Table S1. Accession numbers for the 19 sequences acquired via GenBank.

Table S2. Notes on tip selection process for polyphyletic genera.

Table S3. Fossil taxa and corresponding dates used to calibrate nodes of the starting tree in BEAST. An age of 42 Ma was used for the Baltic amber deposit (Perkovsky et al. 2007, Ward et al. 2015). An age of 15 Ma was used for the Dominican amber deposit (Ward et al. 2015).

Table S5. Binning scheme for all morphological and ecological traits based on categories used in Table S4.

Table S6. Inferred dates of divergence for major clades in our MCC tree, with notes comparing the results of this study to those of previous studies.

Table S7. Notes on the frequency of trait state changes inferred using SIMMAP, averaged across 500 simulations.

Table S8. Contingency tables used for Fisher's Exact Test. For each trait, the trait state at each node with a rate shift inferred in MEDUSA (Table 1, main text) was noted along with the type of shift (rate increase or decrease).

Table S9. Extended results for the phylogenetic tests of correlation between all possible morpho-morpho and eco-morpho trait pairs. Parameter labeling corresponds to parameter designations in BayesTraits (Pagel and Meade 2013, p. 10).