Rise of the spiny ants: diversification, ecology and function of extreme traits in the hyperdiverse genus *Pheidole* (Hymenoptera: Formicidae)

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Study of extreme phenotypic traits and novel structures provides insights into forces mediating evolution and diversification. Sexual selection is often implicated as the evolutionary driver of trait exaggeration, while examples invoking natural selection are scarce. Heretofore overlooked examples include outlandishly exaggerated spines produced by the non-reproductive castes of various ant lineages. Ant spines are conjectured to be defensive weapons, but factors shaping their evolution are poorly understood. Here we assess the evolution, ecology and biomechanics of spine exaggeration in the hyperdiverse genus *Pheidole*. We suggest ant spines are novel structures among Hymenoptera. We ask how many times spinescence evolved in *Pheidole*, the extent to which correlations between spinescence and other morphological traits can inform our understanding of spine function and whether spinescence is associated with evolution into high-elevation habitats. We address these questions by mapping spinescence onto a 145-species phylogeny. We determined that spinescence evolved independently at least seven times in *Pheidole* and that all six extant spinescent lineages are restricted to the Asia-Pacific region. Our results support hypotheses proposing that elongated dorsal spines serve as defence against vertebrate predation and invertebrate attack and that task division is especially pronounced within the worker caste of spinescent species.

ADDITIONAL KEYWORDS: caste – computed tomography – convergent evolution – defensive strategies – exaggerated traits – functional ecology – morphology – novel structures – phylogeny – spines.

INTRODUCTION

Nineteenth-century naturalists advanced evolutionary theory by synthesizing behaviour, form and function with a deepening appreciation for Earth's age. Giraffes stretching for foliage suggested to Lamarck (1809) that this very behaviour caused neck elongation over successive generations. But inference of a structure's function from its form becomes more challenging when divorced of behavioural observation. Using the gargantuan antlers of Irish Elk (*Megaloceros giganteus*) as example, Gould (1974) chronicled a contentious debate between proponents of Darwinism and orthogenesis over various 'bizarre structures' belonging to extinct animals.

The adaptive significance of *Stegosaurus* plates stoked a more contemporary argument, prompting the lamentation that 'bizarre structures' (*sensu* Gould), 'constitute an especially challenging problem to paleobiologists interested in functional morphology (de Buffrenil, Farlow & de Ricqles, 1986)'. Similar sentiments are expressed by students of tropical insects, who find morphologically extraordinary specimens curated in lifeless repose, a world apart from their still breathing prey, predators, competitors and mates.

The 'bizarre structures' of enigmatic function explored here are the dorsal spines of ants – pointy cuticular appendages that protrude, sometimes menacingly, from the upper surfaces of the body (Figs 1, 2). Dorsal spines were such tantalizing characters to early taxonomists that myriad groups were recognized on the basis of their variation. Many such names were since declared invalid

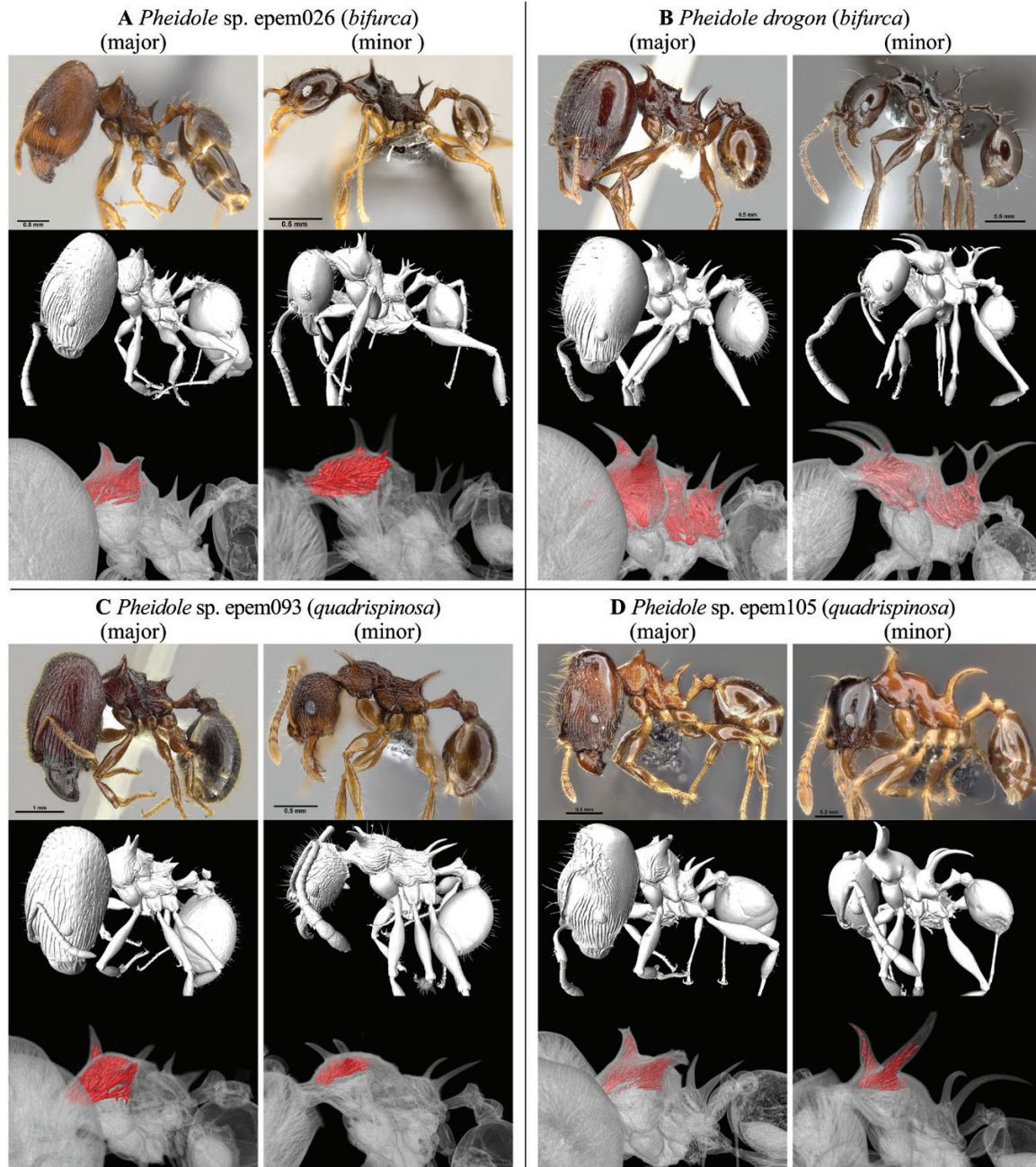*Corresponding author. E-mail: e.sarnat@gmail.com

Figure 1. Illustrations of spinescent *Pheidole*. Specimen photographs (top), micro-CT surface reconstructions (middle) and micro-CT virtual dissections of promesonotal muscle fibres (bottom) are presented for the major and minor worker of each species. Clade names appear in parentheses. Muscle fibres in addition to those of the promesonotum are segmented for scans of *Pheidole drogon*. (A) *Pheidole* sp. epem026, (B) *P. drogon*, (C) *Pheidole* sp. epem093, (D) *Pheidole* sp. epem105. Interim species nomenclature is from AntWeb.org.

as modern taxonomy (Brown, 1973; Bolton, 1995) – and more recently molecular phylogenetics (Sarnat & Moreau, 2011; Ward *et al.*, 2015; Blanchard & Moreau, 2017) – revealed that shape, presence and proliferation of ant spines are less reliable signals of phylogeny than earlier classifications implied.

Ant spines have received remarkably little attention despite repeated evolution across disparate lineages. Outstanding questions concern their developmental origins, adaptive significance and phenotypic extremism. Answers will advance our understanding of how novel structures arise, how multifunctional
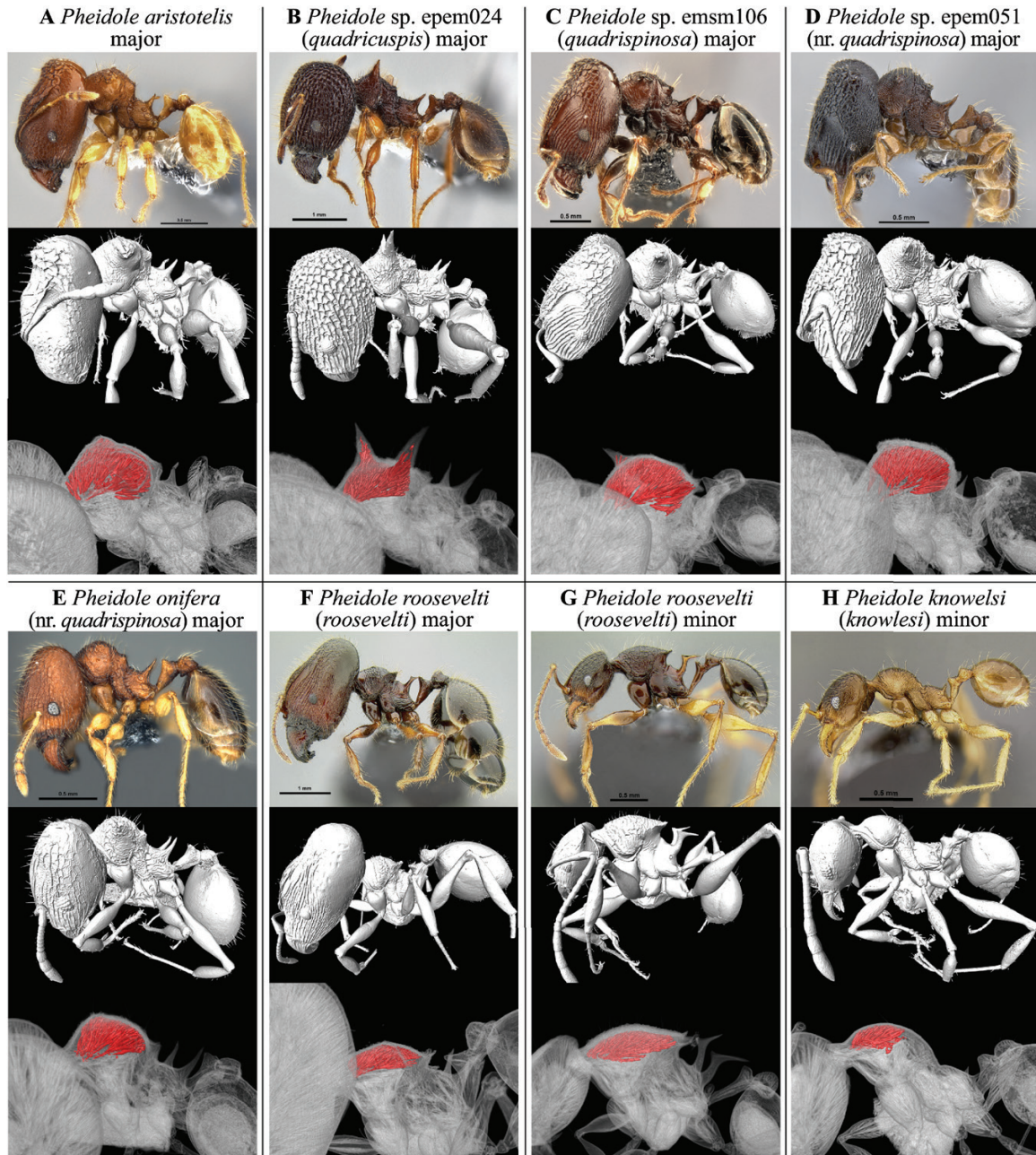
Figure 2. Illustrations of spinescent *Pheidole*. Refer to Figure 1 for additional explanation. (A) *Pheidole aristotelis*, (B) *Pheidole* sp. epem024, (C) *Pheidole* sp. epem106, (D) *Pheidole* sp. epem051, (E) *P. onifera*, (F) *P. roosevelti* major, (G) *P. roosevelti* minor, (H) *P. knowlesi*.

phenotypes evolve and how exaggerated traits are shaped by natural selection. We broadly explore these fundamental questions as they relate to all ants before focusing on the evolutionary history, morphological variation and ecological consequence of spinescence in the hyperdiverse ant genus *Pheidole*.

ANT SPINES AS NOVEL FEATURES

We suspect the dorsal spines of ants to be an important but overlooked morphological innovation in insects. Acknowledging the considerable debate regarding what constitutes novelty in evolution, these armaments satisfy even the stricter criteria required

by contemporary definitions (Müller & Wagner, 1991; Moczek, 2008; Wagner, 2014). Dorsal spines are neither homologues of structures ancestral to ants nor are they serially homologous to existing structures expressed by ants. The progenitor of crown group ants is hypothesized to have lacked spines (Blanchard & Moreau, 2017). Members of the Apoidea – the clade of bees and vespid wasps sister to the ants (Johnson *et al.*, 2013) – also lack any structural precursors to dorsal spines. Lastly, ant spines appear to be appendages evolved *de novo*: although frequently expressed in pairs, they are not modifications or serial copies of some other body part such as legs, wings, antennae or mouthparts.

Dorsal spines were repeatedly gained and lost across the evolutionary history of ants and now occur in 79 of the 334 extant genera (Blanchard & Moreau, 2017). The great majority of spiny ant genera belong to the subfamily Myrmicinae – putatively the only major ant lineage in which spines are plesiomorphic. Whereas most spine losses have occurred within Myrmicinae, gains are mostly scattered across 14 distantly related genera spanning seven subfamilies.

EXTREME MORPHOLOGIES AND TRAIT EXAGGERATION

While the spines of most ants are modestly proportioned, they occasionally achieve an exuberance rivalling the ostentatious displays of ungulate antlers, carnivore sabreteeth or bird plumages (Sarnat, Fischer & Economo, 2016: fig. 1). Extreme morphologies are rampant among insects (Grimaldi & Engel, 2005), and famous examples (Emlen & Nijhout, 2000) include the antlers and eyestalks of tephritid and diopsid flies; the elongated combat legs of bugs (Coreidae), weevils (*Macromerus bicinctus*) and harlequin beetles (*Acrocinus longimanus*); and the distended mandibles of lucanid and cerambycid beetles.

Elongated ant spines are arguably most reminiscent of beetle horns with respect to exaggerated morphological features: both constitute evolutionary novelties, are projecting outgrowths of the cuticle and were gained and lost repeatedly throughout evolutionary time. Whereas beetle horns are the beneficiaries of longstanding interest (Darwin, 1871) and active research (Eberhard, 1977; Pomfret & Knell, 2006; Emlen, Corley Lavine & Ewen-Campben, 2007; Moczek & Rose, 2009), ants spines have only recently received scientific attention (Ito, Taniguchi & Billen, 2016; Sarnat *et al.*, 2016; Blanchard & Moreau, 2017).

The most striking difference between the ornate weaponry of beetles and ants is that the former was forged by *sexual selection* and the latter by *natural selection*. Ants are a noteworthy exception to a near

universal rule – at least for insects (Emlen & Nijhout, 2000) – that exaggerated traits are expressed by males to compete for mates. In contrast, elaborate ant spines are most strongly expressed in the female, non-reproductive worker caste. That these armaments are often diminished in the reproductive female and male castes suggests negligible roles as sexually selected ornaments or weapons.

WHY DO ANTS HAVE SPINES?

Common wisdom holds that elongated dorsal spines defend ants against vertebrate predation by making them more difficult to subdue and ingest (Bequaert, 1922; Buschinger & Maschwitz, 1984; Redford, 1987; Dorow, 1995; Dill, Williams & Maschwitz, 2002; Dornhaus & Powell, 2010; Feldhaar, 2011; Ito *et al.*, 2016; Mezger & Moreau, 2016). In the parlance of predation ecology (Edmunds, 1974), ant spines are considered 'physical weapons of purely defensive function'. Predation is a strong driver of evolution (Vermeij, 1982), and spines have evolved as defensive weapons across a broad range of animals (Edmunds, 1974; Inbar & Lev-Yadun, 2005) – including terrestrial examples such as porcupines (Sweitzer & Berger, 1992), corylid lizards (Losos *et al.*, 2002) and horned lizards (*Phrynosoma*) (Bergmann & Berk, 2012).

Predation gives context to why dorsal spines evolved repeatedly across ants but not their hymenopteran relatives. Faced with fight or flight, ants have but one option. Ants, at least their worker castes, are among the few hymenopteran radiations to lose one of the greatest defence strategies of all: winged escape.

SPINESCENT *PHEIDOLE*

The focal taxa of our study are a subset of *Pheidole* ants (Hymenoptera: Formicidae) that are adorned with elaborate cuticular spines and protruding spikes. These *Pheidole* are so aberrant compared to their less morphologically remarkable relatives that they were grouped together in the subgenus *Pheidolacanthinus* Emery (1921). Although the polyphyly of *Pheidolacanthinus* was eventually confirmed (Sarnat & Moreau, 2011; Economo *et al.*, 2015a), striking phenotypes of several disparate lineages are similar enough to fool even modern taxonomists (Wilson, 1959b; Baroni Urbani, 1995; Sarnat, 2008; Chen *et al.*, 2011).

Pheidole, despite being among the most ecologically diverse, taxonomically rich and geographically ubiquitous ant lineages on earth (Wilson, 2003; Moreau, 2008; Economo *et al.*, 2015a) are not known for morphological invention. A study of New World species found that while *body size* varied considerably, *body shape* remained remarkably invariant, and suggested

that phenotypic constraint left little opportunity for the evolution of specialized morphologies (Pie & Traniello, 2007). Edward Wilson's (1959b) prediction that spinescence is associated with conspicuous foraging and soil nesting was validated by studies of Fiji's *Pheidole* (Sarnat, 2008; Sarnat & Moreau, 2011; Economo & Sarnat, 2012; Sarnat & Economo, 2012; Fischer, Sarnat & Economo, 2016). Unlike their hypothesized common ancestor (Economo & Sarnat, 2012) and non-spinescent living relatives (Fischer *et al.*, 2016), members of the spiny *Pheidole roosevelti* group forage conspicuously and nest directly in stable soil substrates (Sarnat, 2008). Sarnat & Moreau (2011) proposed the elaborate armature of Fiji's spiny *Pheidole* evolved *in situ* from an early colonist lineage as a novel defence against vertebrate predators, which in turn unlocked foraging opportunities unavailable to their more diminutive, litter-dwelling relatives. Economo & Sarnat (2012) expanded on the ecological opportunity hypothesis (Schluter, 2000; Losos, 2010) by arguing that the range shifts to high elevation, loss of dispersal ability and increased ecological specialization observed among Fiji's spinescent *Pheidole* were predicted by the taxon cycle (Wilson, 1959a, 1961).

EVOLUTION, MORPHOLOGY AND ECOLOGY OF SPINESCENT *PHEIDOLE*

The goal of this study was to better understand the causes and consequences of spinescence in ants using comparative analyses of phylogenetic, morphometric and ecological data compiled for *Pheidole*. We asked three central questions. (1) How many times did spinescence evolve and what is the biogeographic distribution of the spinescent lineages? (2) To what extent can correlations between spinescence and other morphological traits inform our understanding of spine function? (3) Is spinescence associated with evolution into high-elevation habitats, as suggested by the single evolution of spinescence in Fijian *Pheidole*? We specifically sought to explain spine elongation and proliferation in *Pheidole* by testing the following five hypotheses.

Vertebrate-defence hypothesis

The first hypothesis proposes that spinescence is a morphological response to shifts in foraging strategy: exposure to vertebrate predation broadens as workers shift from below-ground foraging to above-ground foraging. We tested the vertebrate-defence hypothesis by comparing spinescence with two morphological traits used to indicate foraging strata – relative leg length and relative eye size. Myrmicine foraging strategy can be inferred by measuring the allometric scaling of leg length as body size varies (Kaspari & Weiser, 1999; Weiser & Kaspari, 2006). Proportionally shorter legs increase efficacy of cryptic foraging in the interstitial environments of leaf litter. Proportionally longer legs increase efficacy of conspicuous foraging in the more linear environments of ground surface and vegetation. Eye size has also been shown to increase relative to body size as ants shift from hypogaeic to epigaeic foraging (Weiser & Kaspari, 2006). Reduction in relative eye size is often the consequence of decreased investment in visual processing required for low-light environments, such as leaf litter (Gronenberg & Hölldobler, 1999; Bulova *et al.*, 2016). If conspicuous foraging strategies drive spine elongation and proliferation for vertebrate defence, we predicted spinescence to increase as a function of longer legs and larger eyes.

Invertebrate-defence hypothesis

The second hypothesis proposes that propodeal spines protect ants from invertebrate attack. Whereas outward projecting spines are thought to mitigate vertebrate predation, spines projecting over important articulation points are thought to defend against invertebrate enemies (Dornhaus & Powell, 2010). The precariously slender petiole is one such vital connection point. We therefore tested the invertebrate-defence hypothesis by comparing spinescence with petiole elongation. If the posteriorly projecting propodeal spines of *Pheidole* protect against gaster dismemberment by invertebrate enemies, we predicted that their length should increase relative to petiole elongation.

Task-division hypothesis

The third hypothesis proposes that spinescence is associated with greater task differentiation between individuals in the same colony. Considerable research has explored whether worker polymorphism within ant colonies improves whole-organism performance (*sensu* Irschick *et al.*, 2008) by facilitating divergent phenotypes specialized for distinct ecological functions (Wilson, 1980, 1983; Hasegawa, 1993a, b; Billick, 2002; Powell, 2008; Mertl & Traniello, 2009; Powell, 2009). With regard to caste evolution, there is both empirical and theoretical evidence for the coupling of morphological specialization in association with resource specialization (Planqué *et al.*, 2016).

A defining character of *Pheidole* – and one that has been cited to explain its hyperdiversity (Wilson, 2003) – is the discrete dimorphism of the non-reproductive caste into smaller minor workers (minors) and larger major workers (majors or soldiers). Minors perform general tasks such as brood care, nest maintenance and resource foraging; majors specialize in colony defence,

food processing and food storage (Wilson, 2003). The massive mandibles of *Pheidole* majors serve as biological husking knives and millstones for processing seeds (and other arthropods) too hard for minor workers to crush (Whitford *et al.*, 1981; Hölldobler & Wilson, 1990; Ferster, Pie & Traniello, 2006; Moreau, 2008; Pirk, di Pasquo & Lopez de Casenave, 2009). Seeds are important resources not only for desert dwelling *Pheidole* (Whitford *et al.*, 1981; Wilson, 2003) but also for tropical rainforest species (Roberts & Heithaus, 1986; Kaspari, 1993; Levey & Byrne, 1993; Passos & Oliveira, 2003).

We tested the task-division hypothesis by comparing spinescence with the head width difference between majors and minors – a trait previously associated with subcaste specialization in *Pheidole* (Mertl, Sorenson & Traniello, 2010) and seed-based diet specialization in particular (Holley *et al.*, 2016). An examination of *Pheidole* with dimorphic and trimorphic worker castes associated increases in head size with broader and less serrated mandibles, larger head muscle volume and greater bite force (Huang, 2012). Moreover, while head *elongation* increases mandible speed and is associated with predatory ants, head *widening* increases mandible force and occurs in granivorous ants such as *Pogonomyrmex* – and presumably *Pheidole* – that crack seeds (Paul & Gronenberg, 1999; Paul, 2001). If spinescence is specifically associated with granivory, we predicted that a disproportionate number of spinescent species would be documented as harvesting seeds and maintaining nest granaries. If ecological specialization is highly pronounced between the majors and minors of spinescent *Pheidole*, we predicted that spinescence would increase as a function of worker polymorphism as measured by differences in head width.

Head-support hypothesis

The fourth hypothesis proposes that certain dorsal spines also serve biomechanical functions. The head-support hypothesis (Sarnat *et al.*, 2016) suggests that the most novel phenotypic feature of spinescent *Pheidole* – the pronotal spines – first evolved as skeletomuscular adaptations for supporting the disproportionately large heads of the majors, and were only subsequently selected for elongation in the minors in response to vertebrate predation. Strong correlations between head width and pronotal width reported for New World *Pheidole* majors (Pie & Traniello, 2007) also suggests biomechanical constraint in generating sufficient support for their massive head. If pronotal spines of majors increase the weight-bearing capacity of neck muscles, we predicted that majors with larger heads should produce proportionally larger spines.

High-elevation hypothesis

The fifth hypothesis proposes that spinescence is associated with high-elevation habitats. This hypothesis was motivated by the elevational distribution of Fiji's spinescent *Pheidole* (Sarnat, 2008; Sarnat & Moreau, 2011; Economo & Sarnat, 2012; Sarnat & Economo, 2012) and anecdotal observations by E.M.S. and colleagues familiar with New Guinea's *Pheidole* fauna (M. Janda, P. Klimes, pers. observ.). Although elevation alone is unlikely to affect spinescence, it is plausible that aspects intrinsic to high-elevation habitats such as particular predators, food resources or forest structure might select for spine elongation and proliferation. In Fiji, the transition to spinescence was associated with shifts to upland habitats and ecological specialization and interpreted as a latter phase in the taxon cycle hypothesis (Wilson, 1959a, 1961; Economo & Sarnat, 2012). However, because both spinescence and high-elevation habit only evolved once, there is no evolutionary replication. Here we test if spinescence is associated with high-elevation habitat using the multiple evolutions of spinescence in the genus.

In this study, we assess the evolutionary history, ecological interactions and biomechanical implications of dorsal spine elongation and proliferation in *Pheidole* ants. We map continuous measures of spinescence onto a 145-species phylogeny to reconstruct the evolutionary origins and biogeographic patterns of spinescence across all major lineages of Old World *Pheidole*. We test how spinescence relates to defensive strategy, foraging strategy, caste polymorphism and elevational distribution using phylogenetically controlled analyses of morphometric and ecological data. The elongated dorsal spines of ants are widely considered physical weapons of purely defensive function. Here we investigate an alternative hypothesis proposing spines might also serve as skeletomuscular adaptations for relaxing biomechanical constraints imposed by the unusually large heads of soldier ants. Considering the dorsal spines of ants are neither ancestrally nor serially homologous with respect to Formicidae or any of its hymenopteran relatives, their study could yield valuable contributions to our understanding of evolutionary developmental biology. And because spine elongation and complexity reach their zenith in non-reproductive females, rather than mate-seeking males or queens, spinescent ants represent a unique model for studying how natural selection can drive phenotypic extremism. This study can therefore support future work on developmental mechanisms that generate novel structures in insects, and help identify evolutionary and ecological processes that drive exaggeration of morphological traits unaffected by sexual selection.

METHODS

DEFINING SPINESCENCE

With respect to ants, we define spinescence as the elongation and proliferation of dorsal spines. Dorsal spines are acuminate cuticular appendages protruding from the upper surfaces of the head, thorax (pronotum and mesonotum) and first two to three abdominal segments (propodeum, petiole and postpetiole).

MORPHOMETRICS

We tested hypotheses correlating spinescence with external morphology by recording measurements of 145 *Pheidole* species using linear morphometrics, landmark analysis and vector path length analysis. Criteria for taxon inclusion were based on Old World *Pheidole* for which both phylogenetic and morphometric data were available.

Linear morphometrics

Eleven linear morphometric measurements (Fig. 3, Table 1) were recorded for minor workers (145 specimens) and major workers (88 specimens). We sampled multiple individuals for each subcaste when such data were available. Our average sampling effort was 2.9 specimens for minor workers and 2.4 specimens for major workers. All measurements with the exception of femur length were recorded from Antweb.

org specimen photographs (see Economo *et al.*, 2015a) using tpsDig software version 2.22 (Rohlf, 2007). Femur length could not be accurately measured from standard view photographs and was instead recorded from dry-mounted specimens using an x–y stage micrometer and a Leica MZ16 dissecting stereoscope.

Landmark analysis

We used two-dimensional geometric morphometric methods to describe overall body shape and size for *Pheidole* species, following methods described in Economo *et al.* (2015a). Landmarks were placed on standardized specimen photographs from head (13 landmarks) and profile (6 landmarks) views (Supporting Information, Figs S3–S11). To ensure consistency of measurements, all landmarks were placed by a single researcher, B.L. Landmarks were aligned using a generalized Procrustes analysis in the R v.3.3.3 package *geomorph* v.3.0.3 (Adams & Otárola-Castillo, 2013). We assigned the scaling factor of the profile landmarks as an estimate of body size and used this estimate to correct for allometric relationships throughout this study (hereafter: *body size*).

Path length analysis

We broadly define spinescence as the elongation and proliferation of dorsal spines. For the purposes of our analyses of *Pheidole* ants, we use two more specific
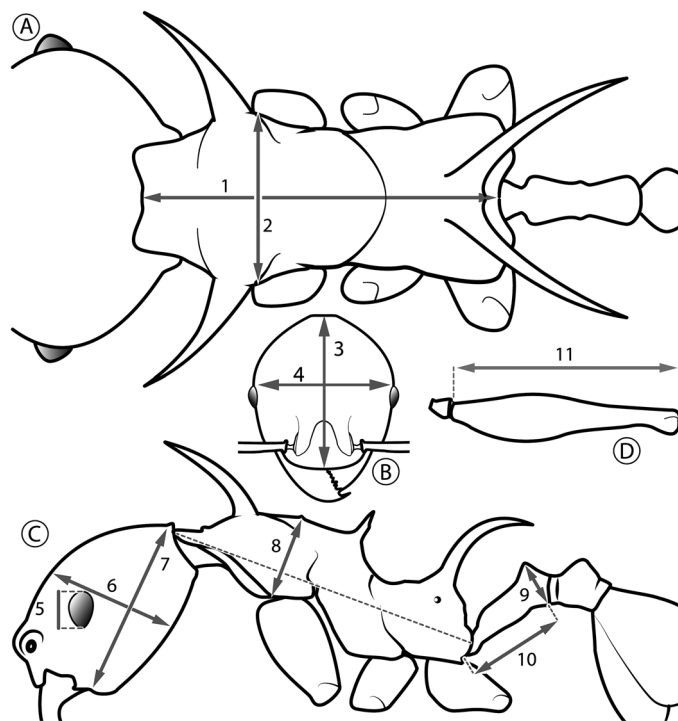

Figure 3. Linear measurements used for morphological analyses. (A) Dorsal view, (B) face view, (C) profile view, (D) anterior view. Numbers refer to terms in Table 1.

Table 1. Measurement terms and abbreviations used for morphological analysis arranged by label number or abbreviation

Label	Abbreviation	Term
1	ML	Mesosoma length
2	PMW	Promesonotal width
3	HLf	Head length (face view)
4	HWf	Head width (face view)
5	EL	Eye length
6	HDp	Head depth (profile view)
7	HLp	Head length (profile view)
8	PMH	Promesonotal height
9	PetH	Petiole height
10	PetL	Petiole length
11	FL	Metafemur length
–	BPL	Body path length
–	EI	Eye index = (EL corrected with *body size*)
–	FI	Femur index = (FL/HWf × 100)
–	HWDiff	Head width difference (HWf$_{major}$ – HWf$_{minor}$)
–	MnSPL	Mesonotal spine path length
–	PrnSPL	Pronotal spine path length
–	PrpSPL	Propodeal spine path length
–	PMW2	Total promesonotal width = (PMW + 2 × PrS)
–	*PSI*	*Propodeal spine index* = PrpSPL/(BPL × 100)
–	*TSI*	*Total spine index* = (PrnSPL + MnSPL + PrpSPL)/(BPL × 100)

Labels refer to numbers in Figure 3. En dash (–) indicates terms that are not labelled in Figure 3.

definitions. The first is categorical and defines as spinescent those species in which pronotal spines occur in the worker caste. Our sampling for *Pheidole* minors with pronotal spines was based on the review of 1428 species and morphospecies imaged on Antweb.org, relevant literature and Old World specimens from our personal collections and those of the Australian National Insect Collection (Canberra, Australia), Museum of Comparative Zoology (Cambridge, USA), Philip S. Ward Collection (Davis, CA, USA) and United States National Museum of Natural History (Washington, DC, USA).

We also treated spinescence as a continuous character corrected for body size using morphometric indices. Spine indices were measured as the proportion of mesosoma perimeter scored as spine multiplied by 100. We measured spine indices for minor workers representing 145 putative species by rendering the mesosoma profile of specimen photographs from raster to vector format using Adobe Illustrator CC, and calculated path lengths using the paths feature. Terms and abbreviations for path length measurements are presented in Table 1.

The entire profile path length is defined as *body path length*. Those portions of the profile path coded as propodeal and mesonotal spines were isolated and their lengths defined separately. Propodeal spines were defined as beginning at the point directly dorsal (0°) to the propodeal spiracle and ending at the point directly posterior (90°) to the propodeal spiracle. Mesonotal spines were defined as any portion of the mesonotum forming an acute angle. Pronotal spines were defined as any protrusion breaking the profile outline of the promesonotum. All path lengths defined as spines were scored red in the mesosomal profile outlines presented in Fig. 4. Pronotal spines, which often diverge from the profile outline, were not included in body path length but were instead measured separately as *pronotal spine path length*. Two indices were calculated from the path length measurements: *total spine index* (*TSI*) and *propodeal spine index* (*PSI*). Although our two-dimensional perimeter analysis of the mesosoma profile accurately captures spinescence occurring along the anterior–posterior and dorsal–ventral axes, it is inadequate for measuring spinescence occurring along the lateral axis. Although marginal, this bias systematically undervalues spinescence to the extent spines project laterally.

PHYLOGENY AND COMPARATIVE METHODS

We used a phylogeny including 145 *Pheidole* species for comparative analyses. The tree is a chronogram inferred for a previous study on *Pheidole* biogeography in the Old World (Economo *et al.*, 2015b). To test hypotheses of correlated evolution between traits, we used comparative methods that accounted for phylogenetic non-independence. As data were not always available for all species in each analysis, we included only the species for which data were available.

We used a phylogenetic generalized least squares 1989 (PGLS; Grafen, 1989) framework for each analysis with a lambda correlation structure estimated from the data (Pagel, 1999). When predictor variables were uncorrected for body size prior to the analysis, we corrected for allometric relationships by log-transforming variables and including log-transformed *body size* as a covariate (see Freckleton, 2009).

FUNCTIONAL ECOLOGY OF SPINESCENCE

Morphological predictors

To infer the functional role of dorsal spines in defending foragers against vertebrate predation, we compared *TSI* with two indicators of epigaeic foraging strategy: relative leg length (*femur index*) and relative eye size (*eye index*). In separate analyses, we compared *TSI* to the interaction of both *femur index* and *eye index* with a categorical variable identifying the spinescent lineages (hereafter *spinescent clade*). To infer the functional role
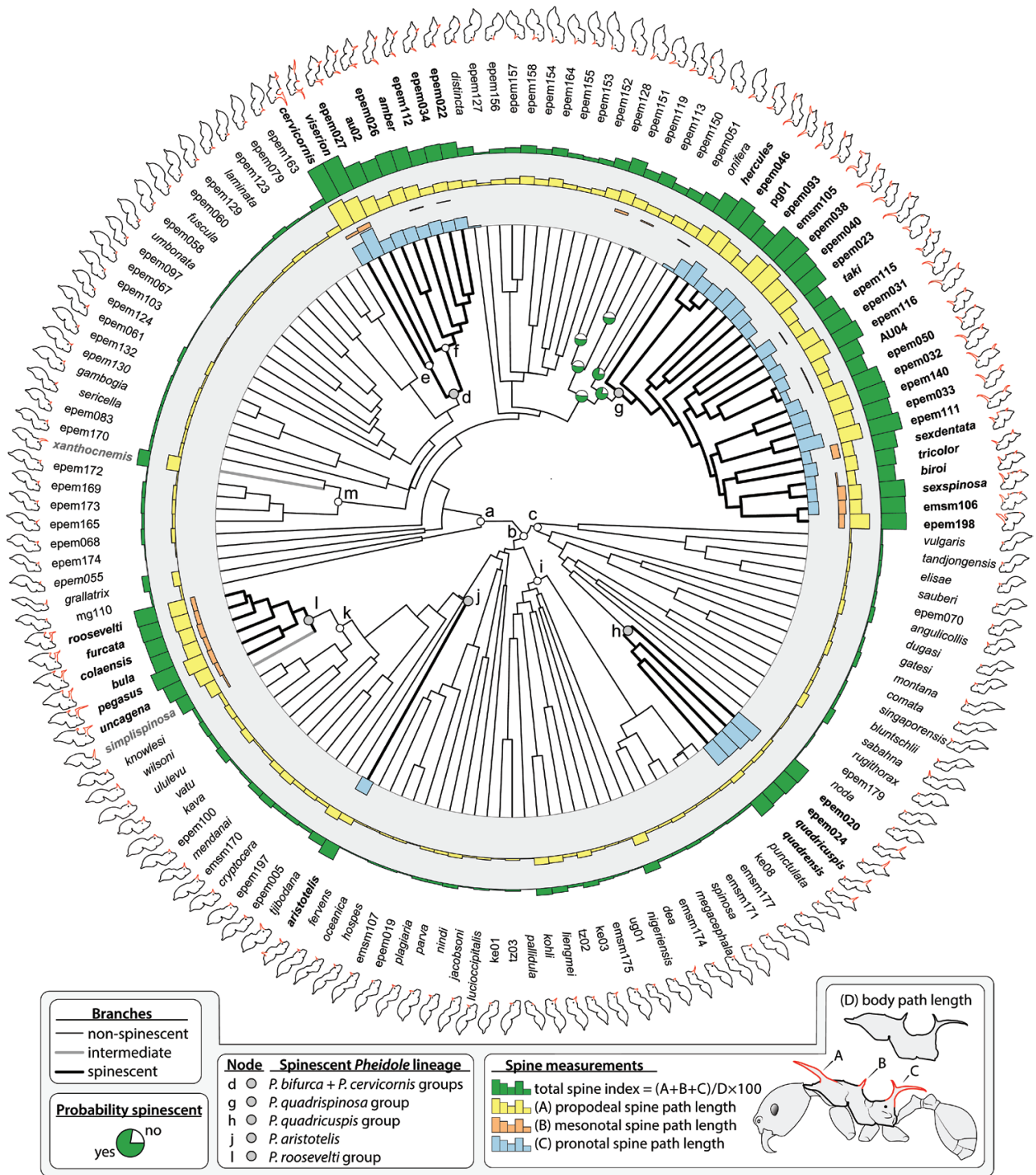
Figure 4. Phylogenetic distribution of spinescence across Old World *Pheidole*. Letters (a–j) label clades and correspond to names presented in Table 3. Thick black branches denote clades with spinescent morphotypes. Thick grey branches denote intermediate morphotypes. Nodes with unknown ancestral states are denoted with pie charts (green = spinescent, white = not spinescent). Coloured histograms refer to *TSI* (green), and the measured path lengths of propodeal spines (yellow), mesonotal spines (orange) and pronotal spines (blue). Illustrations of body perimeters are presented for each species with path lengths scored as spines coloured red.

of spines in defending against invertebrate enemies, we analysed the relationship between *PSI* and *petiole length* (corrected for *body size*). We tested the extent to which spinescence is associated with worker dimorphism by comparing *TSI* to the calculated difference between major and minor head widths (*DiffHW*) (Holley *et al.*, 2016). In a separate analysis, we compared *TSI* to the interaction of *DiffHW* and *spinescent clade*. To infer the functional role of pronotal spines in reducing biomechanical constraints of head support for majors, we analysed the relationship between *total promesonotal width* and two measures of head size: *head width* and *head length* (all of which were corrected for *body size*).

Ecological predictors

We explored the relationship between spinescence and elevation by comparing *TSI* to *elevation*, both directly and as an interaction between *elevation* and *island*. We accounted for the differences in geographic variables and community composition within the bioregion by conducting separate analyses for the *Pheidole* fauna of Borneo, Fiji and New Guinea. Species occurrence records were compiled from the Antweb and GABI (Guénard *et al.*, 2017) databases. Elevation values were estimated using data from the Shuttle Radar Tomography Mission (Hijmans *et al.*, 2005), then averaged for each species. Some ant species may inhabit a broad range of elevations, which is a potential limitation of this approach (see Supporting Information, Fig. S2).

EVOLUTIONARY MODELS OF SPINESCENCE

We compared Ornstein–Uhlenbeck models of spine length evolution among spinescent and non-spinescent taxa, and tested whether a single-optimum or multi-optimum model better fit our spinescence (*TSI*) data in OUwie v.1.50 (Beaulieu *et al.*, 2012).

SPINESCENCE IN MORPHOSPACE

We tested for an overall spinescent syndrome with respect to body size and shape by comparing spinescence (*TSI*) in a multivariate model including shape principle components (PCs) as predictors. PCs were calculated based on the relative warping of head and mesosoma shape to describe geometric variation in those features (Supporting Information, Figs S4–S11). Finally, to derive general estimates of body shape, we combined the first two PCs from head and mesosoma shape with six of the measurements (*mesosoma width*, *head length (profile)*, *head depth*, *pronotum height*, *petiole length* and *petiole height*) in a phylogenetically corrected PC analysis using *phytools* v.0.6-00 (Revell, 2012). The analysis was conducted separately for minor and major workers.

SPINESCENCE AND GRANIVORY

We sought direct evidence for relationships between spinescence and granivory by compiling ecological data on seed caching and analysing morphological features predictive of highly granivorous *Pheidole*. Data associated with *Pheidole* from Australasia, Indomalaya and Oceania were collected by reviewing our own field observations, searching relevant literature (Eguchi, 2001, 2008; Eguchi, Yamane & Zhou, 2007) and querying the Antweb.org database for granivory associated terms in the fields 'behaviour' and 'collection note'.

MICROTOMOGRAPHIC ANALYSIS

We investigated the extent to which dorsal spines serve as skeletomuscular adaptations for head support using micro-computer tomographic (micro-CT) methods and equipment outlined in Fischer *et al.* (2016) and Sarnat *et al.* (2016). We assessed the extent to which thoracic muscles used for head support extended into internal cavities of dorsal spines. Assessed muscles included the dorsal promesonotal muscles that attach to the posterior cranium and the lateral portions of the propleura. Muscle fibres were manually segmented with Amira version 6.2 using unprocessed dicom scan files to facilitate qualitative morphological analysis.

RESULTS

MORPHOLOGICAL ANALYSIS OF SPINESCENCE IN *PHEIDOLE*

Results of our morphological analysis revealed that *Pheidole* dorsal spines are confined to three segments of the mesosoma: the propodeum, mesonotum and pronotum. Propodeal spines, while reduced in many species, are plesiomorphic in *Pheidole* and occur in workers and queens. Pronotal spines are apomorphic in *Pheidole* and evolved at least six times in the minor worker subcaste including four independent radiations (*bifurca*, *quadrispinosa* and *quadricuspis* clades) and two single-species origins (*Pheidole aristotelis* and *Pheidole hainanensis*). Pronotal spines also occur in two Dominican amber fossil species, *Pheidole tethepa* and *Pheidole primigenia* (Wilson, 1985; Baroni Urbani, 1995).

Quantifying spinescence using *TSI* recovered values ranging from 4 to 48 and *PSI* values ranged from 4 to 30. The distribution of *TSI* across the observed species is bimodal, with the major mode between 5 and 10, the minor mode between 25 and 30, and the antimode between 15 and 20 (Fig. 5). We treat as spinescent all taxa (102 spp.) with *TSI* for minors above 20. Species with *TSI* below 15 (47 spp.) are treated as nonspinescent. The three species with *TSI* between 15 and 20 (*Pheidole simplispinosa*, *Pheidole xanthocnemis* and *Pheidole* sp. epem113) are treated as transitional.

Propodeal spine length, measured either linearly or as *PSI*, tends to positively correlate with *TSI* (PGLS: $P < 0.001$ for both comparisons). A notable exception is the *quadricuspis* clade, which is distinguished by strongly reduced propodeal spines but elongated pronotal spines. Only species of the Fijian *roosevelti* group lack pronotal

Figure 5. Histogram of *TSI* for 145 *Pheidole* minor workers. The two spinescence optima occur at *TSI* = 8.4 and *TSI* = 32.5.

spines but have *TSI* > 20. Although our path length analysis accurately captured spinescence occurring along the anterior–posterior and dorsal–ventral axes, it is less accurate at measuring spinescence occurring along the lateral axis. This bias undervalued spinescence to the extent spines projected laterally. Despite limitations, the *TSI* values agreed with our a priori concepts of relative spinescence among the observed species.
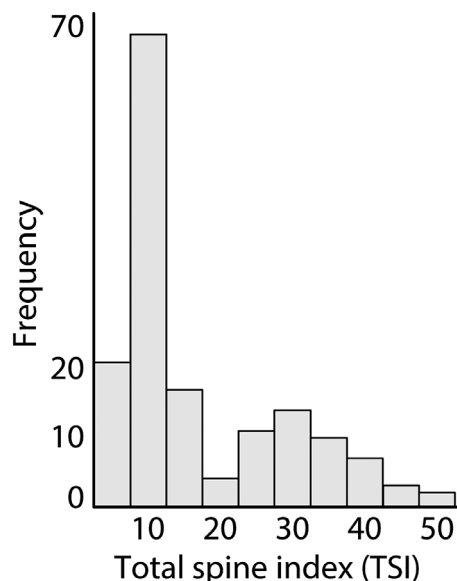
PHYLOGENETIC AND BIOGEOGRAPHIC PATTERNS OF *PHEIDOLE* SPINESCENCE

Spinescence, defined either by the presence of pronotal spines or *TSI* > 20, evolved independently at least seven times in *Pheidole*: six times in extant lineages restricted to the Asia-Pacific region (Indomalaya, Australasia and Oceania), and at least once in an extinct clade known only from Dominican amber (Figs 4, 6; Tables 2 and 3). The *quadricuspis* group (Eguchi *et al.*, 2016) is a modest radiation of mostly insular species nested within a broader Indomalayan clade. *Pheidole leloi*, a putatively relictual species discovered on the east coast of Vietnam (Eguchi *et al.*, 2016), is the only *quadricuspis* group member endemic to continental Asia. Aside from *P. leloi* and three widespread species known from peninsular Malaysia and its northern border, the remainder of the *quadricuspis* group are endemic to Borneo, the Philippines, and Indonesia. The spinescent *P. hainanensis* (Chen *et al.*, 2011) was originally assigned to the *quadricuspis* group on the
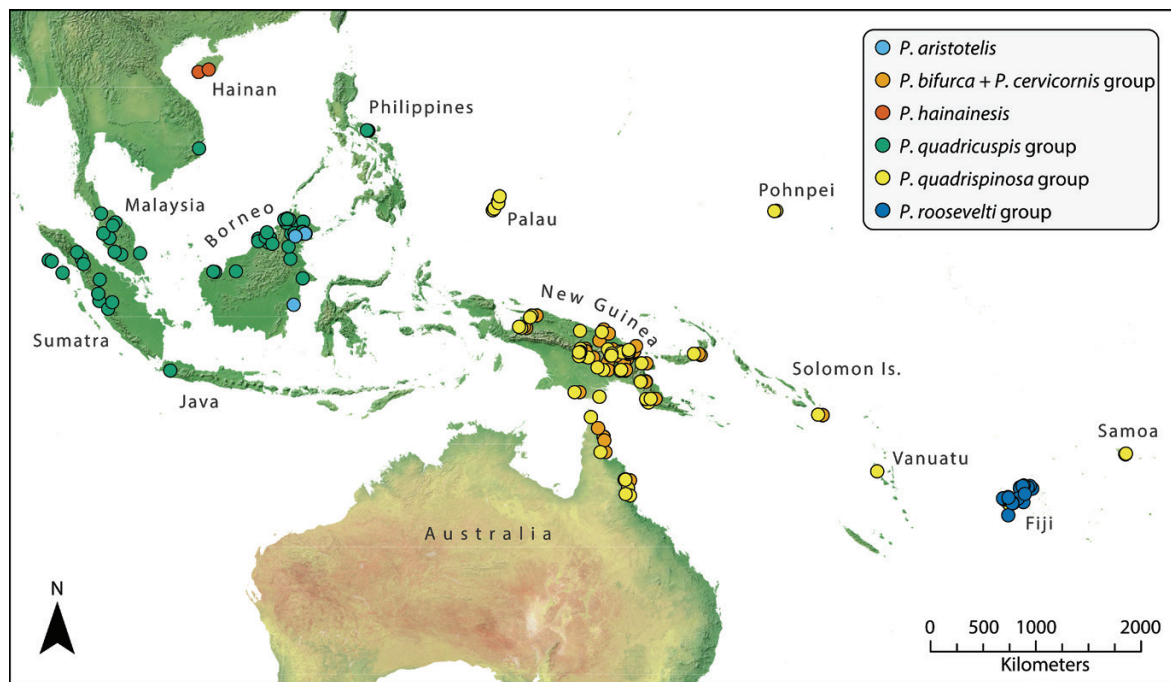

Figure 6. Distribution of extant spinescent *Pheidole* lineages. Overlapping symbols are offset.

Table 2. Spinescent *Pheidole*

Clade	Distribution	Age (Mya)	Species
aristotelis	Borneo	–	1
bifurca	Australasia	12	48 (5)
hainanensis	Hainan (China)	–	1
quadricuspis	Indomalaya	10	9 (7)
quadrispinosa	Australasia	8	74 (14)
roosevelti	Fiji	5	7
*tethepa**	Dominican amber	15	2

Divergence dates for extant crown group radiations as approximated from Economo *et al.* (2015a) are presented in Mya. Spinescent species are presented as total number of putative species followed by the number of valid species in parentheses.
*Extinct lineage.

Table 3. Clades and species groups labelled in the phylogeny of Old World *Pheidole* (Fig. 4)

Node	Clade/taxon
a	Australia–New Guinea clade
b	Asia-Africa clade
c	Southeast Asia clade
d	*bifurca* clade
e	*cervicornis* group
f	*bifurca* group
g	*quadrispinosa* group
h	*quadricuspis* group
i	Africa clade
j	*Pheidole aristotelis*
k	Fiji clade
l	*roosevelti* group
m	New Caledonia clade

basis of its pronotal spines. Although molecular data were unavailable, our morphological analysis suggests *P. hainanensis* is more closely related to *Pheidole nodus*, and the pronotal spines are homoplastic with respect to those of the *quadricuspis* group.

The *quadrispinosa* group represents an Australasian radiation of over 70 putative species, 14 of which are described. The group nests within a broad clade of New Guinean *Pheidole*. At least two lineages of the *quadrispinosa* group colonized the Australian wet tropics, and one species of the *sexspinosa* complex is endemic to the remote island of Vanuatu. *Pheidole sexspinosa* Mayr is reported from islands across Oceania and is probably expanding its range in association with anthropogenic dispersal (Sarnat *et al.*, 2015). All members of the group produce elongated propodeal and pronotal spines. The most basal lineages, represented by species closely related to *Pheidole hercules*, tend towards gigantism. The smaller workers of the *quadrispinosa* and *sexspinosa* complexes produce

distinctive majors characterized by deeply excavated antennal scrobes and concave head vertices.

The *bifurca* clade is an Australasian radiation of the reciprocally monophyletic but deeply diverged *bifurca* and *cervicornis* groups. Many *bifurca* group species are known only from older museum collections and are underrepresented in this study. Preliminary morphological analysis suggests the group consists of over 40 putative species, only one of which is described. Additionally, the diversity of spine length and shape within the group exceeds that of any other *Pheidole* lineage. Pronotal spines range from nearly vestigial to extremely long, and propodeal spines range from short and simple to long and bifurcated. Furthermore, the hypostomal bridge dentition – usually a conserved trait – is highly variable. The *cervicornis* group, represented by four species endemic to New Guinea, is the most spinescent *Pheidole* lineage. The species all possess strongly bifurcated propodeal spines, well-developed mesonotal spines and long pronotal spines. *Pheidole cervicornis* is the only *Pheidole* to produce bifurcated pronotal spines.

The *roosevelti* group belongs to a larger radiation of Fijian endemics and is composed of seven species distributed across the archipelago's high-elevation forests. It is the only spinescent *Pheidole* lineage to lack pronotal spines. *Pheidole simplispinosa*, which lacks bifurcated propodeal spines and well-defined mesonotal projections, is considered an intermediate morphotype (*TSI* = 17). The minor workers of the remaining species, which together form a clade sister to *P. simplispinosa*, all possess strongly bifurcated propodeal spines and well-developed mesonotal projections.

Pheidole aristotelis is restricted to Borneo, and its minor workers are the only member of the *hortensis* group with elongate pronotal spines. The pronotum of the major worker extends into a pair of broad horns, but are too obtuse to be considered spines.

MORPHOLOGICAL AND ECOLOGICAL PREDICTORS OF SPINESCENCE

We found a significant positive correlation between spinescence (*TSI*) and relative leg length (*femur index*; $\lambda = 0.98$; $n = 144$; $P < 0.001$, Table 4). When *spinescent clade* was included as an interaction term in the model, the relationship remained significant and positive for four of five lineages ($\lambda = 0.98$; $n = 144$; *P. aristotelis*: $P = 0.09$, *bifurca* group: $P < 0.01$, all others: $P < 0.05$). However, in this model, we did not observe a significant relationship between leg length and spine size for species scored as non-spinescent ($P = 0.47$). As predicted by the vertebrate-defence hypothesis, ants belonging to spinescent lineages had longer legs relative to their body size when compared to non-spinescent clades (Fig. 7B). Contrary to the prediction of the vertebrate-defence hypothesis, spinescent species tended to have

Table 4. Results of phylogenetic generalized least squares fit analyses

Predictor variable	Effect size	SE	*T*-value	*P*-value	Response variable
Leg length (FI), minor*	18.99	7.21	2.63	0.0094	TSI
non-spinescent	4.01	5.50	0.73	0.4672	TSI
bifurca clade*	16.57	5.38	3.08	0.0025	TSI
quadricuspis clade*	12.36	6.01	2.06	0.0416	TSI
quadricuspis clade*	12.58	5.56	2.26	0.0252	TSI
roosevelti clade*	11.90	5.37	2.21	0.0285	TSI
Head width difference (HWDiff)**	8.06	2.34	3.45	0.0009	TSI
non-spinescent	1.87	1.64	1.14	0.2573	TSI
bifurca clade**	11.41	1.78	6.40	< 0.0001	TSI
quadricuspis clade**	8.12	2.26	3.59	0.0006	TSI
quadricuspis clade**	13.03	2.16	6.02	< 0.0001	TSI
roosevelti clade**	8.90	1.93	4.62	< 0.0001	TSI
Petiole length (PetL), minor + body size**	55.74	7.33	7.61	< 0.0001	PSI
Eye size (EL), minor + body size	−18.28	9.47	−1.93	0.0556	TSI
Elevation	0.00	0.00	1.52	0.1312	TSI
Borneo	0.00	0.00	0.37	0.7115	TSI
Fiji	0.00	0.00	1.32	0.1876	TSI
New Guinea*	0.00	0.00	2.40	0.0175	TSI
Other	0.00	0.00	0.21	0.8316	TSI
Head width (HW)	0.28	0.32	−0.88	0.3837	PrW2
Head length (HL)	−0.30	0.34	−0.89	0.3745	PrW2

*$P < 0.05$, **$P < 0.001$.

proportionally smaller eyes rather than larger ones. We found a negative but non-significant association between *TSI* and *eye size* after accounting for phylogeny and *body size* ($\lambda = 0.93$; *n* = 145; $P = 0.056$) (Fig. 7D). Conspicuous foraging was confirmed by field observations for all of Fiji's *roosevelti* group species (Sarnat, 2008) and for members of the Australasian *bifurca* and *quadrispinosa* clades (E. M. Sarnat, pers. observ.).

We observed a strong positive relationship between petiole length and propodeal spine length ($\lambda = 0.93$; *n* = 145; $P < 0.001$). As predicted by the invertebrate-defence hypothesis, ants belonging to spinescent clades had longer petioles relative to their body size when compared to non-spinescent clades (Fig. 7C). The one exception was the *quadricuspis* group, which produces elongated pronotal spines but truncated propodeal spines.

We found no significant relationship between *total promesonotal width* and either *head width* or *head length*. Contrary to the prediction of the head-support hypothesis, majors with pronotal spines did not have proportionally larger heads than majors lacking pronotal spines.

We found a significant positive correlation between spinescence (*TSI*) and worker polymorphism (*DiffHW*;

$\lambda = 1.00$; *n* = 88, $P < 0.001$). When *spinescent clade* was included as a covariant in the model, we observed a relationship with *DiffHW* that was both significant and positive in its direction within all five spinescent lineages ($\lambda = 1.00$; *n* = 88; *P. aristotelis*: $P < 0.05$, all others: $P < 0.001$). In contrast, we observed no such relationship among species scored as non-spinescent in this model ($P = 0.26$). As predicted by the task-division hypothesis, spiny species produce large major workers relative to their minor workers after accounting for phylogeny and body size (Fig. 7A, Table 4).

A univariate comparison of average elevation and spinescence showed no significant relationship between these two traits ($\lambda = 0.99$; *n* = 148; $P = 0.13$), contrary to the high-elevation hypothesis. However, a multivariate model using *island* as an interaction term showed a significant relationship for species inhabiting New Guinea ($P < 0.05$), but not other islands (all others: $P > 0.05$) (Fig. 8).

EVOLUTIONARY MODELS OF SPINESCENCE

Our results indicate that a model allowing multiple trait and rate optima was the best fit to our data (77% Akaike information criterion weight). This model
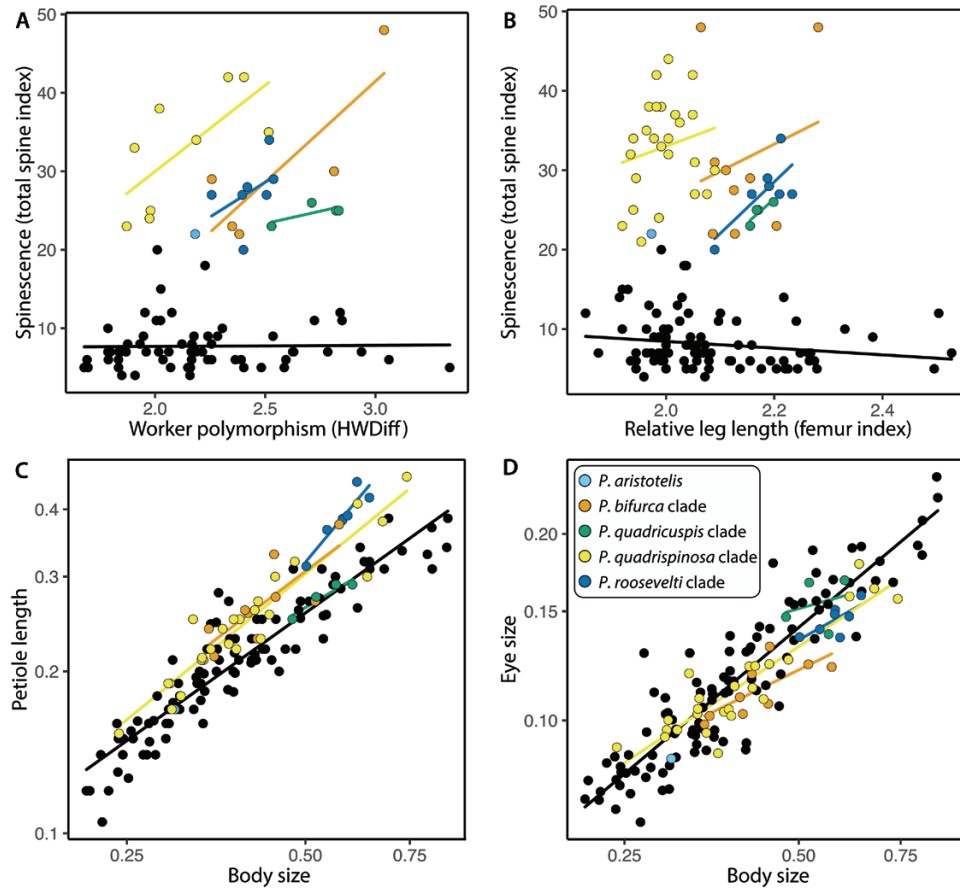
Figure 7. Morphological predictors of spinescence. Spinescent *Pheidole* clades are coded by colour: light blue = *Pheidole aristotelis*, orange = *bifurca* clade, green = *quadricuspis* clade, yellow = *quadrispinosa* clade, dark blue = *roosevelti* clade. Black symbols represent non-spinescent Old World *Pheidole*. (A) Spinescence (*TSI*) vs. worker polymorphism (*HWDiff*). (B) Spinescence (*TSI*) vs. relative leg length (*femur index*). (C) Petiole length vs. *body size*. (D) Eye size vs. *body size*.

described two spinescence optima, at *TSI* = 8.4 and *TSI* = 32.5 (Fig. 5).

SPINESCENCE IN MORPHOSPACE

Our phylogenetic PCA of *Pheidole* morphological traits yielded six PCs that together accounted for greater than 98% of trait variance; this was observed for both minors and majors. *Body size* was the first PC for both subcastes, with all morphological measurements loaded together strongly in the same direction; subsequent PCs described aspects of body shape (Tables 5 and 6). We then compared these PCs together against *TSI* in a multivariate model accounting for phylogeny (minors: λ = 0.99, *n* = 145; majors: λ = 1.00, *n* = 88). Our results for minors show significant relationships between *TSI* and PC1 (body size; *P* < 0.001), PC2 (mesonotum depth; *P* < 0.001) and PC6 (mesonotum shape; *P* < 0.05). Similarly, our results for major workers show significant

relationships between *TSI* and PC1 (body size; *P* < 0.001), PC3 (mesonotum depth; *P* < 0.05) and PC4 (pronotum width; *P* < 0.01).

SPINESCENCE AND GRANIVORY

Our review of *Pheidole* species from the Asia-Pacific region documented as caching seeds in their nests are presented in Supporting Information, Table S1. Although additional species were recorded as taking seeds in the field opportunistically, either from naturally occurring sources or from artificial baits, we excluded these records to identify species for which seed harvesting is most important. Half (7/14) of the *Pheidole* species recorded with granaries were spinescent. The two groups with the highest incidence of granaries were the spinescent *quadricuspis* group (four spp.) and seven species referred to here as the *hortensis* group, of which only *P. aristotelis* is spinescent.
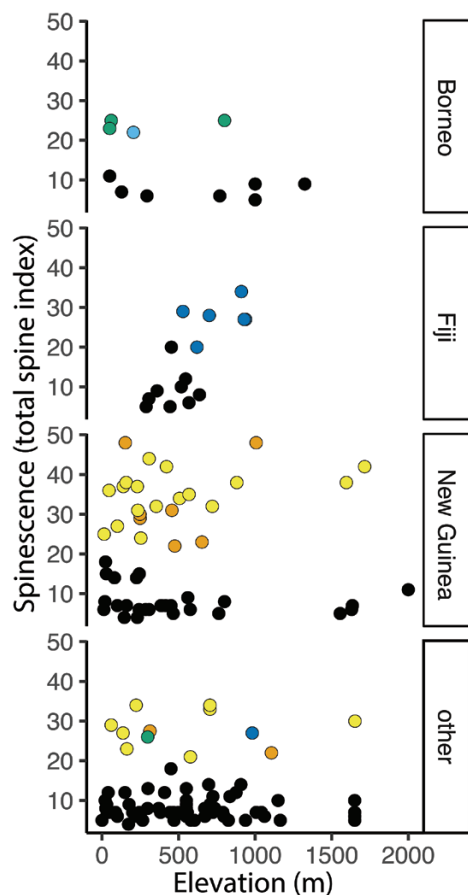
Figure 8. Spinescence (*TSI*) vs. average elevation for *Pheidole* species from the islands of Borneo, Fiji and New Guinea. All species included in our study not recorded from the aforementioned islands are plotted under 'other'. Spinescent *Pheidole* clades are coded by colour: light blue = *Pheidole aristotelis*, orange = *bifurca* clade, green = *quadricuspis* clade, yellow = *quadrispinosa* clade, dark blue = *roosevelti* clade. Black symbols represent non-spinescent Old World *Pheidole*.

MICROTOMOGRAPHIC ANALYSIS

We completed x-ray micro-CT scans for 21 specimens representing majors and minors of nine *Pheidole* species. Virtual dissections of the most informative specimens are presented (Figs 1, 2). Complete volumetric and specimen data are available from the authors upon request.

Analysis of the micro-CT scans of spinescent *Pheidole* clades revealed a diversity of promesonotal musculature patterns. Majors of *P. aristotelis* (Fig. 2A), *Pheidole onifera* (Fig. 2E) and *Pheidole* sp. epem051 (Fig. 2D) produce a broadly extended dorsolateral pronotum packed with indirect neck muscles. Minors of *P. aristotelis* produce short, mostly hollow pronotal spines, while those of the latter two lack pronotal spines. Majors of species in the *bifurca* (Fig. 1A, B), *quadrispinosa* (Figs 1C, D, 2C) and *quadricuspis* (Fig. 2B) groups produce elongate pronotal spines. The basal half or more of these pronotal spines are usually filled with muscle fibres while the distal portions remain hollow. Minors of these groups show varying degrees of neck muscle extension into pronotal spines: (1) *P. onifera*, *Pheidole* sp. epem024, *Pheidole* sp. epem051 and *Pheidole* sp. emsm106 produce hollow pronotal spines without visible muscles; (2) *Pheidole drogon* and *P. aristotelis* show only a single muscle strand shallowly reaching into the base of the pronotal spines; and (3) *Pheidole* sp. emsm105 produces extremely elongate pronotal spines that are filled with muscles. Unlike the laterally oriented neck muscles of the aforementioned species, those of *P. roosevelti* are oriented posteriorly. In comparison to the neck muscles of non-spinescent *Pheidole*, including the closely related *Pheidole knowlesi* (Fig. 2H), those of the *P. roosevelti* minor worker conspicuously extend towards a thickly produced mesonotal process (Fig. 2G). In contrast to the highly conserved dome-shaped promesonotum characteristic to non-spinescent *Pheidole* minor

Table 5. PCA loadings for minor workers

Trait	PC1	PC2	PC3	PC4	PC5	PC6
ML	−0.989	0.100	0.087	0.006	−0.027	0.021
PrW	−0.943	−0.168	−0.001	0.077	0.186	0.042
HDp	−0.924	−0.092	−0.220	−0.156	0.133	−0.186
HLp	−0.956	−0.142	−0.146	−0.004	−0.175	−0.045
PMH	−0.864	−0.341	0.198	0.062	0.058	−0.001
PetH	−0.838	−0.099	−0.114	0.122	0.253	−0.022
PetL	−0.880	−0.209	0.024	−0.008	0.168	0.240
HshapePC1	0.239	−0.392	0.136	−0.792	0.004	0.218
HshapePC2	0.392	0.085	0.158	−0.478	−0.292	0.363
MshapePC1	0.386	−0.867	0.079	0.140	−0.112	0.033
MshapePC2	−0.279	0.182	−0.785	0.123	−0.016	0.443

Table 6. PCA loadings for major workers

Trait	PC1	PC2	PC3	PC4	PC5	PC6
ML	−0.961	0.263	0.072	−0.023	−0.035	0.031
PrW	−0.953	0.007	−0.088	−0.202	0.009	−0.191
HLp	−0.984	−0.169	0.051	−0.015	−0.005	0.026
HDp	−0.983	0.026	−0.078	0.154	0.025	−0.049
PMH	−0.903	0.068	−0.298	−0.104	−0.001	0.017
PetL	−0.846	0.090	−0.294	−0.076	0.308	0.088
PetH	−0.919	−0.053	−0.130	−0.156	0.049	−0.031
HshapePC1	−0.108	0.030	−0.171	−0.153	0.479	0.370
HshapePC2	0.130	−0.358	0.510	−0.073	−0.020	−0.221
MshapePC1	−0.269	−0.186	−0.683	−0.005	−0.570	0.155
MshapePC2	−0.382	−0.031	0.271	0.063	−0.099	−0.507

workers (e.g. Fig. 2H), the promesonotal dorsum of the *P. roosevelti* minor is highly modified into a shield-like surface composed of extremely thick cuticle.

DISCUSSION

EVOLUTIONARY AND BIOGEOGRAPHIC HISTORY OF SPINESCENCE IN *PHEIDOLE*

Pheidole ants show extreme diversity, but comparatively little morphological disparity. Since canalization of ancestral worker polymorphism fixed on discrete major and minor subcastes, morphological innovation has mostly been consigned to size scaling (Pie & Traniello, 2007; Pie & Tscha, 2013; Economo *et al.*, 2015a; Holley *et al.*, 2016). The conservative, weakly armed phenotype hypothesized for Neotropical stem-group *Pheidole* persisted in the first Old World colonist lineage, and was inherited by its descendants that invaded Europe, Africa, Asia and Australia. While each new invasion triggered the rapid evolution of a broad spectrum body sizes (Economo *et al.*, 2015a), the standard *Pheidole* blueprint held constant with few exceptions [e.g. the aberrant Australian *Anisopheidole* and *Machomyrma*, both now synonymized with *Pheidole* (Ward *et al.*, 2015)]. But it was only when *Pheidole* colonists reached the islands of Indomalaya and Australasia that lineages evolved elaborate dorsal weaponry.

Pronotal spines – an innovation previously limited to a now extinct lineage of Caribbean *Pheidole* – most likely evolved separately in the ancestors of one Indomalayan and two Australasian radiations, and twice in unrelated species from otherwise non-spinescent lineages. The extent to which pronotal spines of each lineage resulted from novel genetic process or instead from a latent developmental potential retained from a common ancestor, as in the case of *Pheidole* supermajors (Rajakumar *et al.*, 2012), remains unknown. Some degree of homology

is probable, however, considering 11 of the other 13 pronotal-spined ant genera are myrmicines, and of these seven are also attines (*sensu* Ward *et al.*, 2015) including the highly spinescent cephalotine ants (*Cephalotes* + *Procryptocerus*) that are sister to *Pheidole* (Ward *et al.*, 2015; Branstetter *et al.*, 2017).

Drawing on the spandrel and side effect hypotheses (Gould & Lewontin, 1979; Müller, 1990), we speculate that selection for pronotal spine elongation in *Pheidole* minors was preceded by selection for lateral pronotum expansion in majors: the same developmental pathway that originally relieved biomechanical constraints in the major caste was later co-opted to produce elongated spines in the minor caste. This pathway first led to specialized skeletomuscular architecture for food processing or colony defence in the majors and subsequently led to specialized anti-predation weaponry for improving foraging efficiency in the minors.

FUNCTIONAL ECOLOGY OF SPINESCENT *PHEIDOLE*

From a deterministic vantage, independent evolution of spinescence across distantly related *Pheidole* lineages suggests spine elongation and proliferation evolved in response to shared ecological opportunities and challenges. Our study tested four hypotheses concerning the causes and consequences of spinescence in *Pheidole*. We stress that while comparative analyses are useful for identifying correlation, they cannot address causation (Powell & Franks, 2006). Experimental studies will be needed to address the causal relationship between spinescence and the morphological, behavioural and ecological traits discussed below.

Vertebrate-defence hypothesis and foraging strategies

The disproportionately long legs of spinescent *Pheidole* suggests selection for defensive weaponry in species

that have evolved conspicuous foraging. What advantages might elongate spines confer upon above-ground foragers? One potential advantage is that spinescence decreases the risk of conspicuous foraging by reducing predation by vertebrates (Buschinger & Maschwitz, 1984; Redford, 1987; Bequaert, 1922; Dorow, 1995; Dill *et al.*, 2002; Dornhaus & Powell, 2010; Feldhaar, 2011; Ito *et al.*, 2016; Mezger & Moreau, 2016). While epigaeic foraging might increase foraging efficiency – perhaps through greater resource access and faster discovery rates – it also increases exposure to natural predators such as mammals, birds, reptiles and amphibians.

That the elaborate armature of ants evolved in response to vertebrate predation pressure might seem self-evident. Specifically *how* ant spines deter vertebrates is, however, poorly known. Physical defence and aposematic defence are two candidate – and possibly complementary – mechanisms. If a predator injures its mouthparts or digestive system by ingesting a spiny forager, it might spit out that individual or desist from capturing additional foragers. And to the extent the predator associates injury with the visual cues of prominent spines, it might learn to avoid spiny ants in the future.

Experimental support for the negative effect of ant spinescence on vertebrate predation is limited to a single study evaluating the defensive functions of *Polyrhachis* spines on *Hyla* tree frogs (Ito *et al.*, 2016). The authors found that elongated petiolar spines protected workers from predation and that frogs learned to recognize this prey as unpalatable. The curved and bifurcated spines of the *Pheidole bifurca*, *quadrispinosa* and *roosevelti* clades might also confer better predation deterrence than linear spines to the extent they hook into predators' throats.

Elaborate spines serve as aposematic warnings for numerous plants. Aposematic spines are less prevalent among animals and are rare even among terrestrial arthropods (Inbar & Lev-Yadun, 2005). Although spines, unlike poisons, serve as their own self-advertising warning signals, the coevolution of auxiliary visual cues such as bright or contrasting coloration can enhance the cost-effectiveness of antipredator defences (Speed & Ruxton, 2005). Unlike certain *Polyrhachis* lineages (Pekár *et al.*, 2017), the coincidence of aposematic colours with spine ornamentation is rare among spinescent *Pheidole*, suggesting coordinated displays are either ineffective or developmentally constrained.

If spinescence is a response to conspicuous foraging, we also expected spiny ants to produce proportionally large eyes. Contrary to our prediction, we found instead that spinescent *Pheidole* tend to be proportionally small-eyed. Although olfaction – rather than visual perception – is the predominant sensory modality in

Pheidole (Ilieş, Muscedere & Traniello, 2015), could the negative association between spinescence and eye size suggest spiny species are better adapted to the low-light environments of the leaf litter than those of the surface? In their study of New World ants, Weiser & Kaspari (2006) found that eye size relative to body size generally increases as ants shift from below-ground foraging to above-ground foraging. However, the relationship was non-significant for the myrmicomorph clade to which *Pheidole* belongs (Weiser & Kaspari, 2006). Moreover, a recent analysis of defensive strategies across ant genera reported a positive relationship between evolutionary transition rates of spinescence and eye size (Blanchard & Moreau, 2017). Here too phylogenetic resolution – and trait resolution – deserve consideration. The study analysed 268 ant genera and categorized traits as binary (e.g. spines of any size present vs. absent; eyes with ≤ 10 vs. ≥ 11 ommatidia). *Pheidole*, as example, was treated as a single datum and coded as large eyed and spinescent. Large eyes were discussed in terms of increasing predator evasion, but as ant collectors and photographers will attest, only genera with exceptionally large eyes (≥ 500 ommatidia, e.g. *Gigantiops*, *Harpegnathos*, *Myrmecia*, *Opisthopsis*, *Psuedomyrmex*, *Tetraponera*) respond visually to vertebrate threats.

We propose the relationship found between spinescence and eye size reflects a transition from an underground to above-ground ecology (*sensu* Lucky *et al.*, 2013), rather than a correlation between the two defensive strategies of predator defence (spines) and predator avoidance (large eyes). We do not a priori expect selection pressures mediating trait correlation among genera to scale across species of a single genus. We are unaware of any *Pheidole* species that evade myrmecologists using visual cues, and we are sceptical that the truncated spines common in many ant genera – including most *Pheidole* – serve equivalent functions as elongated spines.

Another explanation for why spinescent *Pheidole* might produce proportionally small eyes involves the potential relationships between habitat type and navigation strategy. While large eyes increase navigation efficacy for desert ants that use path integration, landmarks and skylight compasses (Wehner, 2003), the poor visibility, closed canopy and maze-like understories of rainforests are likely to select for alternative strategies, such as olfaction (Shashar *et al.*, 1998; Ehmer, 1999; Rodrigues & Oliveira, 2014). Whereas the proportionately large-eyed species included in our analysis mostly occur in the open habitats of interior Australia and eastern Africa, spinescent *Pheidole* predominantly inhabit tropical rainforests. The *sexspinosa* complex is perhaps the exception that proves the rule: they are both the largest-eyed species of the

quadricuspis clade and the most disturbance tolerant of any spinescent *Pheidole* lineage. Similarly, within the Fijian clade, the large-eyed species are more tolerant of open habitats, while the spinescent species are restricted to closed-canopy rainforests (Sarnat, 2008; Economo & Sarnat, 2012; Fischer *et al.*, 2016).

Invertebrate-defence hypothesis

The worst enemies of ants are not vertebrates, but other ants (Hölldobler & Wilson, 1990). Spinescence – and propodeal spinescence in particular – is also thought to protect against invertebrate attack (Dornhaus & Powell, 2010). The strong positive correlation we found between propodeal spinescence and petiole length suggests that these spines might protect ants from each other during interspecific and intraspecific warfare by shielding their precariously slender waists from dismemberment. The functional consequences of petiole elongation are largely unexplored. Elongated petioles allow greater articulation of the gaster, which can be important for defence, subduing prey or applying pheromone trails crucial for the group foraging and defensive strategies used by *Pheidole* (Wilson, 2003). An alternative explanation is that the petioles of *Pheidole* assist the gaster in counterbalancing the weight of the major worker's head (Pie & Traniello, 2007). Of the four spinescent clades, only the *quadricuspis* group lacks elongated petioles (Fig. 7C). The *quadricuspis* group is also the only spinescent clade with short propodeal spine and derives its high *TSI* from elongated pronotal spines. When *PSI* is measured instead of *TSI*, the correlation with petiole length increases, suggesting that petiole elongation is not associated with spinescence per se, but with propodeal spine elongation specifically.

Task-division hypothesis

We tested the hypothesis that spinescence is related to task division within the worker caste by comparing the intraspecific difference in worker polymorphism. Our results supported the prediction that spinescence is significantly more pronounced in lineages with high worker dimorphism. Specifically, our results found that *Pheidole* with increased worker dimorphism are more likely to be spinescent than *Pheidole* with reduced worker dimorphism. What selective pressures might drive selection for spinescence among strongly dimorphic *Pheidole*? One explanation is increased task division (Ratnieks & Anderson, 1999). Perhaps the extra-wide heads of spinescent *Pheidole* are ecologically specialized for some combination of food processing (e.g. Holley *et al.*, 2016) and colony defence (e.g. Mertl *et al.*, 2010), while the exaggerated spines of the minors provide specialized physical defence against enemies encountered during foraging.

If a direct relationship exists between spinescence and granivory, we would expect corroborating evidence from ecological observation. Regrettably, direct observations of Asia-Pacific *Pheidole* are extremely sparse. The discovery of seed caches in four of the nine known *quadricuspis* group species suggests at least one spinescent clade to be highly granivorous (Supporting Information, Table S1). Field observations and nest excavations are required to more thoroughly decipher relationships between spinescence and diet.

Head-support hypothesis

Our hypothesis that spinescence relates to the head size of majors was tested by comparing the combined promesonotal width and pronotal spine length with head width and length. The analysis did not provide significant support for our prediction that pronotal spine length, in combination with promesonotal width, affects head size. While a more complex analysis – such as a volumetric measurement of promesonotal muscle fibres – might more accurately test the head-support hypothesis, it is certainly conceivable that pronotal spines do not relax biomechanical constraints on head size. For example, most species of *Pheidole* lack pronotal spines, as do some myrmicines with large-headed majors such as *Adlerzia*, *Atopomyrmex* and *Messor*.

However, there is a strong overlap within Myrmicinae between genera that produce pronotal spines and those with strongly polymorphic worker castes. Of the 140 myrmicine genera analysed by Blanchard & Moreau (2017), 9% contain species with pronotal spines and 11% produce large-headed majors. [Blanchard & Moreau, 2017 scored *Aphaenogaster*, *Crematogaster* and *Pheidole* as lacking pronotal spines. However, species with elongated pronotal spines occur in all aforementioned genera.] Half of these polymorphic genera (7/15) contain species with pronotal spines, and half of those with pronotal spines (7/12) are polymorphic.

The results of our microtomographic scans also offer compelling evidence for an association between pronotal spines and the neck musculature of *Pheidole* workers. These first thoracic (T1) muscles are modified in the worker caste of ants to enable the lifting and carrying of objects many times heavier than themselves (Keller, Peeters & Beldade, 2014). The discovery that T1 muscle fibres extend deep into pronotal spines of *Pheidole cervicornis* group major workers – but not into those of minor workers – prompted Sarnat *et al.* (2016) to suggest these spines might have originally evolved as skeletomuscular adaptations in the major subcaste for relaxing biomechanical constraints on head size.

The microtomographic analyses of our current study reveal a largely congruent muscle pattern among species of the two other extant *Pheidole* clades with

pronotal spines: (1) the pronotal spines of the majors are packed with muscle fibres, (2) the pronotal spines of the minors are mostly hollow and (3) the propodeal spines of both majors and minor are mostly hollow (Figs 1, 2). The one exception to this pattern was *Pheidole* sp. epem105 (Fig. 2D), in which the pronotal spines of both the major and the minor are conspicuously muscled. Further study is required to determine whether the condition of *Pheidole* sp. epem105 is exceptional among spinescent minors, occurs more broadly or is an artefact of specimen preservation.

The novelty of hymenopteran pronotal spines begs the same question of all novel structures: how did they first appear when such characters cannot be selected for prior to their existence? Microtomographic scans of three species illustrate how selection for defensive weapons in minors could be an evolutionary side effect (*sensu* Gould & Lewontin, 1979) of selection for increased neck muscles in majors. While pronotal spines are truncated in minors of *P. aristotelis*, they are lacking in majors. However, the shoulders of the major's promesonotum protrude conspicuously, and the entire cavity is packed with muscle (Fig. 2A). A similar condition is observed in majors of *P. onifera* (Fig. 2E) and *Pheidole* sp. epem051 (Fig. 2D), and the minors of these species entirely lack pronotal spines. That *P. onifera* and *Pheidole* sp. epem051 are recovered as sister to the highly spinescent *quadrispinosa* clade suggests the expanded pronotal shoulders evolved in the majors, perhaps as biomechanical adaptations, before becoming weaponized in the minors.

High-elevation hypothesis

Even when the confounding variables of geography and community composition were controlled by conducting separate analyses for Borneo, Fiji and New Guinea, we found little support for our prediction that spinescent species are over-represented among high-elevation habitats. In Borneo, the average elevations for *P. aristotelis* and the *quadricuspis* group species included in our analysis all ranged between low- and mid-elevation habitats. In Fiji, the spinescent *P. roosevelti* group species appear to occur at higher average elevations than most of their non-spinescent congeners, but the sample size is too small to recover any significant relationship. A significant positive result was recovered for New Guinea *Pheidole*, but here the weakness of the relationship suggests elevation alone is a poor predictor of spinescence.

WEAPONIZED SPINES: A GREAT INNOVATION OR FAILED TECHNOLOGY?

If elongated dorsal spines are effective weapons for *Pheidole*, why are they so rare – even among surface foragers? Specifically, how do we explain the extinction of one New World spinescent phenotype, the lack of extant spinescent lineages in the New World and why the spinescent radiations of the Old World are limited to Asia-Pacific islands? We explore two of many possible explanations. The first invokes developmental constraint; perhaps most *Pheidole* lack the developmental pathways required for spine proliferation and elongation. Even if developmental constraints do contribute to the rarity of spinescent *Pheidole*, the repeated multiplication of dorsal spines across independent *Pheidole* lineages and closely related genera suggests additional factors are involved. As evidenced by the 'weird wonders' of the Cambrian Burgess Shale, phenotypic novelties can be evolutionary failures (Erwin & Valentine, 2013). From this perspective, the rarity of spine elongation and proliferation in *Pheidole* could be explained as an opportunity cost: alternative anti-enemy defences have proved more effective across evolutionary time than spine elongation and proliferation. The effectiveness of defensive armour depends on the extent to which it reduces vulnerability to predators relative to the degree to which it reduces an organism's ability to avoid predation by other means (Losos *et al.*, 2002).

The association between spinescence and islands supports both the developmental constraint and opportunity cost propositions. Islands are famous innovation incubators (Lack, 1940; Gillespie & Roderick, 2002; Losos, 2010) and evolutionary graveyards: examples of 'upstream colonization', in which insular endemics colonized large islands or continents are scarce (Filardi & Moyle, 2005; Balke *et al.*, 2009; Clouse *et al.*, 2015). In this context, it is possible to imagine an escape-and-radiate scenario (Arbuckle & Speed, 2015) in which release from ecological and developmental constraints allows insular *Pheidole* to couple conspicuous foraging strategies with spinescent defence strategies (Sarnat & Moreau, 2011; Economo & Sarnat, 2012; Blanchard & Moreau, 2017). While dorsal armaments might serve *Pheidole* as adequate defence for naive island ecosystems (Gittleman & Gompper, 2001), perhaps alternative innovations – such as larger colonies, better visual processing or fiercer aggression (Dornhaus & Powell, 2010) – are more successful defence strategies for *Pheidole* inhabiting the continental ecosystems of ancestral ranges where competition and predation pressures are more acute (Ricklefs & Cox, 1972).

Disadvantages beset all defence strategies (Lima & Dill, 1990), spines and armour included (Losos *et al.*, 2002). What costs do spine elongation and proliferation impose on ants? Study of New Guinea's inordinately spinescent ant fauna prompted Wilson (1959b) to suggest that long spines hinder the exploitation of ecological niches such as twig nesting and subterranean

foraging. The same costs Kaspari & Weiser (1999) ascribed to long legs – that a larger cross-sectional area limits access to interstitial leaf-litter environments – also apply to long spines. If long spines increase effective body size by limiting the interstices through which individuals can fit, we would expect constraints on spine elongation. Our observation that spines tend to either bend or bifurcate along the anterior–posterior axis after achieving a certain length in proportion to body size suggests that increases in cross-sectional area remain costly even to the most spiny ant species.

The cost of spinescence is reflected in its bimodal distribution among *Pheidole*. Scarcity of intermediate species (TSI = 15–20) and the divergent spinescence optima (TSI = 8.4, TSI = 32.5) suggest transitional forms carry higher fitness costs. This would be true if intermediate spines are more metabolically costly and ecologically restrictive than short spines, but are not long or numerous enough to offset these losses with sufficient gains in predator deterrence. The case of *P. simplispinosa* from Fiji runs contrary to the proposition that intermediate morphotypes incur higher fitness costs. Among the *roosevelti* group, it is the only intermediate morphotype (TSI = 20) yet is the most geographically widespread and numerically abundant.

Are weaponized dorsal spines a losing strategy for *Pheidole* species in the evolutionary competition for survival? If so, they would not be alone: the sting of *Pheidole* is vestigial and lost its defensive function long ago (Blanchard & Moreau, 2017). Although associated with higher diversification rates, dorsal spines were lost twice as often as gained across all ant genera (Blanchard & Moreau, 2017). And the discovery of an extinct spinescent *Pheidole* lineage from an island in the Caribbean (Wilson, 1985; Baroni Urbani, 1995) prompts us to question whether the pattern of spine evolution and loss experienced by ancestral New World *Pheidole* will be recapitulated by the more recent colonists of the Old World. Fiji's spinescent *Pheidole* are mostly single-island endemics restricted to pristine mountaintops, while their non-spinescent relatives are thriving across the archipelago (Economo & Sarnat, 2012).

FUTURE RESEARCH

The repeated evolution of spinescence across distantly related lineages of *Pheidole* and numerous ant genera offers compelling opportunities for elucidating which developmental mechanisms generate novel phenotypic features among eusocial insects (Prud'homme *et al.*, 2011; Rajakumar *et al.*, 2012; Londe *et al.*, 2015) and how selection pressures drive morphological exaggeration (Stern & Emlen, 1999). For example, to what extent are spines governed by regulatory mechanisms that specify appendage architecture across insects (Kojima, 2004)? Of particular interest are three limb patterning genes (*Distal-less*, *dachshund* and *homothorax*) that regulate horn expression in *Onthophagus* beetles (Moczek & Rose, 2009). Answering *why* spinescence evolved across multiple lineages, however, will probably require dedicated fieldwork. Many landmark studies of phenotypic convergence in the animal world benefitted from broad knowledge of natural history (Liem & Osse, 1975; Losos *et al.*, 1998; Gillespie, 2004; Mahler *et al.*, 2013). The basic ecology of spinescent ants is comparatively unknown. Measuring effects of dorsal spine elongation and proliferation on enemy deterrence using experiments similar to those designed by Ito *et al.* (2016) would also benefit the study of spinescent ants. Lastly, we suggest using microtomography to test relationships between dorsal spines and biomechanical constraints for other spinescent ant genera.

CONCLUSION

We propose that dorsal spines are a novel feature among Hymenoptera that evolved as *de novo* defensive weaponry to compensate for loss of winged escape. The dramatic elongation of ant spines is an exceptional case of morphological trait exaggeration because it is most strongly expressed in non-reproductive females and is driven by natural selection, not sexual selection. We infer that spinescence evolved at least seven times in *Pheidole* and that all extant spinescent species are restricted to the Asia-Pacific region. We conclude that correlations between spinescence and other morphological traits can be used to explain spine function. Our results are consistent with hypotheses proposing that elongated dorsal spines serve as defence against vertebrate predation and invertebrate attack and that task division is especially pronounced between the worker subcastes of spinescent species. We found little evidence that spinescence is associated with high-elevation habitats. Based on results of our microtomographic analysis, we propose that selection for pronotal spine elongation in *Pheidole* began as selection for neck muscle expansion in the major subcaste and only subsequently for anti-predation weaponry by the minor subcaste. Although our results suggest spinescence has important consequences for foraging strategy, defensive strategy, ecological specialization and biomechanics, we emphasize the need for field observations and behavioural experiments to test these inferences more thoroughly.

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SUPPORTING INFORMATION

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

Table S1. *Pheidole* species from Asia Pacific region documented as caching seeds in nests, arranged by species group. Asterisks indicate spinescent species. Em-dash (—) indicates same value of preceding record. EMS, JTL and PSW numbers refer to collection events of Eli M. Sarnat, John T. Longino and Philip S. Ward.
Figure S2. Comparison of elevation to spine length. Elevation values represent each species' entire range of observed locality elevations.

Figure S3. Illustration of head and profile landmarks (red dots) used for morphometric analysis.
Figure S4. Head of major, PC1. Warp grids with landmarks are presented in the corners.
Figure S5. Head of major, PC2. Warp grids with landmarks are presented in the corners.
Figure S6. Profile of major, PC1. Warp grids with landmarks are presented in the corners.
Figure S7. Profile of major, PC2. Warp grids with landmarks are presented in the corners.
Figure S8. Head of minor, PC1. Warp grids with landmarks are presented in the corners.
Figure S9. Head of minor, PC2. Warp grids with landmarks are presented in the corners.
Figure S10. Profile of minor, PC1. Warp grids with landmarks are presented in the corners.
Figure S11. Profile of minor, PC2. Warp grids with landmarks are presented in the corners.