



## Subcaste-specific evolution of head size in the ant genus *Pheidole*

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An organism's morphology is constrained by its evolutionary history and the need to meet a variety of potentially competing functions. The ant genus *Pheidole* is the most species-rich ant genus and almost every species has a dimorphic worker caste (a few are trimorphic). This separation of workers into two developmentally distinct subcastes (smaller minors and larger majors with distinctively large heads) may partially release individuals from functional constraints on morphology, making *Pheidole* an ideal genus for addressing questions on the evolution of morphology in relation to ecological specialization. Major workers can perform a variety of tasks, although they are usually specialized for defence, as well as food retrieval and processing. *Pheidole* species vary in their diet, although many species gather seeds. The major workers mill the seeds using large jaws powered by mandible closer muscles that occupy a large proportion of the head cavity. In the present study, we examined the relationship between seed-harvesting and morphology in *Pheidole*, hypothesizing that majors of seed-harvesting species would have larger heads relative to non-seed-harvesters to accommodate the powerful mandibular muscles needed to mill seeds. By taking a phylogenetically controlled comparative approach, we found that majors of seed-associated *Pheidole* did not have larger heads (width and length) than majors of non-seed-harvesting species. However, the head length of minors (and to a lesser extent head width) was smaller in seed-harvesters. Additionally, we found the difference in head size between majors and minors was greater in seed-harvesting species. These morphological differences in diet, however, were not related to changes in the rate of evolution in either seed-harvesting or non-seed-harvesting lineages. These findings suggest that the morphologies of worker subcastes can evolve independently of each other, allowing colonies with polymorphic workers to specialize on new resources or tasks in ways not possible in monomorphic species. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, 118, 472–485.

**KEYWORDS:** body size – caste – diet – formicidae – *Pheidole* – seed harvesting – worker polymorphism.

### INTRODUCTION

An organism's morphology dictates how it interacts with the abiotic and biotic environment. The size and shape of the head, for example, and its associ-

ated feeding structures can have a strong influence on diet (Miles & Ricklefs, 1984; Wainwright & Richard, 1995; Clifton & Motta, 1998; Grant, 1999; Aguirre *et al.*, 2002; Hulsey & Wainwright, 2002; Marshall *et al.*, 2012), and subsequently influence community structure (Karr & James, 1975; Grant, 1999). Cichlid fish, for example, underwent a recent, rapid adaptive radiation largely as a result of ecological specialization on feeding guilds driven by morphological adaptation of the jaws and teeth (McConnell &

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Lowe-McConnell, 1987; Kocher, 2004). Comparative studies on morphology often reveal a strong phylogenetic signal suggestive of conservation of form and function among related taxa. Historical constraints can limit both the amount of variation and the tempo of morphological evolution in lineages (Harvey & Pagel, 1991; Garland, Harvey & Ives, 1992) and, in turn, drive patterns of coexistence and diversification among taxa (Ricklefs & Travis, 1980; Wainwright & Reilly, 1994; Losos *et al.*, 1998).

In most animals, morphological variation is constrained by the need to meet a variety of functions, including feeding, mating, and dispersal. Insects, however, have circumvented these constraints in several ways. Holometabolous insects undergo complete metamorphosis, thus allowing selection to optimize two separate morphologies. For example, larvae may be specialized for feeding, whereas the adult form may be specialized for mating and dispersal. Similarly, a reproductive division of labour makes social insects a compelling group to use for testing hypotheses about the evolution of eco-morphology (Powell, 2009; Keller, Peeters & Beldade, 2014). Having distinct reproductive and nonreproductive castes may eliminate some morphological constraints or trade-offs faced by solitary organisms. In the majority of ant species, colony reproduction is the primary role of queens, a dedicated reproductive caste. Queens have large abdomens housing developed ovaries for egg production, as well as wings and developed wing muscles for dispersing (Peeters, 1997). The nonreproductive worker caste is flightless and in many cases completely sterile. The worker caste can further specialize into morphological subcastes that differ in size and shape depending on their behavioural roles in the colony. Approximately 13% of ant species possess morphologically variable worker subcastes (Wilson, 1953; Oster & Wilson, 1978). This size variation among workers can manifest itself in a variety of ways. For example, workers can be dimorphic, with body size fitting a bimodal distribution and with each peak representing a discrete worker subcaste [e.g. most species of *Pheidole* (Wilson, 1953), the tropical fire ant *Solenopsis geminata* (Wilson, 1978; Tschinkel, 2013)]. Worker morphology in a colony can also be continuously variable or polymorphic [e.g. the leaf-cutter ant genus *Atta* (Wilson, 1980) or the red imported fire ant *Solenopsis invicta* (Wilson, 1978)]. The evolution of worker polymorphism in ants has been hypothesized to be associated with specialization of foraging and prey capture (Wilson, 1984; Hölldobler & Wilson, 1990; Powell & Franks, 2005, 2006; Ferster, Pie & Traniello, 2006), defence (Rettenmeyer, 1963; Hölldobler & Wilson, 1990; Powell, 2008), food storage (Wilson, 1974; Tsuji, 1990; Lachaud *et al.*, 1992) or task efficiency (Oster &

Wilson, 1978; Bourke & Franks, 1995; Powell & Franks, 2005; Mertl & Traniello, 2009).

Although most ants are generalist omnivores and scavengers, there are many examples of dietary specialization in ants, including preying on specific insect taxa (Peeters & Crewe, 1987; Hölldobler & Wilson, 1990; Brandão, Diniz & Tomotake, 1991; Leal & Oliveira, 1995; Dejean & Evraerts, 1997; Larabee & Suarez, 2014), rearing fungus as food (Mueller *et al.*, 2001), and harvesting seeds (Wheeler, 1910; Davidson, 1977; Brown, Reichman & Davidson, 1979). Seed-harvesting is commonly seen in desert ecosystems where food availability is seasonal (Wheeler, 1910; Brown *et al.*, 1979). Seeds are collected by foraging workers and taken to the nest where seed chaff is removed and the seeds are stored in dedicated nest galleries called granaries. Many seed-harvesting ants have polymorphic workers and worker size is positively correlated with the size of seeds collected and dispersed by ants (Davidson, 1977; Wilson, 1978; Traniello & Beshers, 1991; Kaspari, 1996; Ness *et al.*, 2004; but see also Rissing, 1981; Willott, Compton & Incoll, 2000). Worker dimorphism in particular often occurs in seed-harvesting species (Hölldobler & Wilson, 1990) and it has been suggested that species with bimodal distributions of head shape have evolved a specialized major subcaste to process seeds for food (e.g. the genus *Pheidole*, some *Pogonomyrmex*, and *Solenopsis geminata*: Wilson, 1978; Hölldobler & Wilson, 1990; Tschinkel, 1998; Ferster *et al.*, 2006).

The genus *Pheidole* is ideal for examining questions related to the evolution of eco-morphology for a number of reasons. First, *Pheidole* ranks among the most species-rich of the 324 described ant genera, with over 1000 described species (Wilson, 2003; <http://antcat.org>). Second, although most *Pheidole* are generalist scavengers and insectivores, seed-harvesting has evolved multiple times in this genus (Moreau, 2008) and many are important seed predators. For example, in a Chihuahuan desert ecosystem, *Pheidole* species remove an estimated  $10^8$  seeds  $\text{ha}^{-1} \text{year}^{-1}$ , which is 10 times the amount removed by other seed-harvesting ants (Whitford *et al.*, 1981). Finally, the genus is characterized by a dimorphic worker caste consisting of small 'minors' and larger 'majors', often referred to as 'soldiers'. A few species, however, possess a third worker subcaste called a 'supermajor', an additional 'soldier' subcaste engaging largely in defence and some processing of dead prey objects (Huang & Wheeler, 2011; Huang, 2012). Major and minor workers vary markedly in the size and shape of the head (Fig. 1). Major workers have enlarged heads compared to minor workers, a trait that has earned this genus the common name of 'big-headed' ants. *Pheidole*

majors often specialize in defence, food-handling (including seed husking and milling), and food storage (Whitford *et al.*, 1981; Wilson, 1984; Tsuji, 1990; Detrain & Pasteels, 1992; Lachaud *et al.*, 1992; Dejean *et al.*, 2005; Huang, 2010; Mertl, Sorenson & Traniello, 2010). By contrast, minor workers have a more extensive behavioural repertoire that includes brood care, food collection, storage and distribution, and nest maintenance (Wilson, 1976a, b, 1984; Brown & Traniello, 1998). Major task repertoire varies depending on the ratio of minors to majors, with majors performing most tasks occasionally (especially when minors are experimentally removed), and repertoires vary extensively among species (Wilson, 1984; Mertl *et al.*, 2010; Sempo & Detrain, 2010; McGlynn, Diamond & Dunn, 2012).

Previous research into the relationships between morphological characters of *Pheidole* has demonstrated that morphological variation in majors and minor can be attributed mainly to shifts in size (Pie & Traniello, 2007). There was little dissociation between morphological features indicating strong constraints on morphological evolution, despite *Pheidole* species occupying diverse niches (Pie & Traniello, 2007). The head morphology of majors did display some dissociation from that of minors, however, which was attributed to specialization in tasks such as seed-milling or defence (Pie & Traniello, 2007). In majors with specialized roles, head morphology may be driven, at least partially, by the size of the mandible closer muscle. The mandibular closer muscle is responsible for generating bite force and its size and function correlates with external head

morphology, particularly the width of the head (Paul & Gronenberg, 1999; Paul, 2001; Huang, 2012).

In the present study, we examined the eco-morphology of minor and major workers of New World species in the genus *Pheidole*. We took advantage of a recently published phylogeny of 140 *Pheidole* species (Moreau, 2008) to investigate how major and minor worker head size and shape have evolved in relation to each other and to diet. Given that worker polymorphism has been associated with seed-harvesting in some genera, we hypothesized that seed-harvesting species of *Pheidole* will have a more pronounced size difference between major and minor workers. Furthermore, if the major subcaste is specialized for seed-milling, we predicted greater differences in the shapes of the head between majors and minors of seed-harvesting species. This would concur with the previous finding that majors and minors have different head allometry, and the dissociation could be attributed to task specialization of majors (Pie & Traniello, 2007). Furthermore, we predicted that a dedicated major subcaste specialized to mill seeds will require more strength to break seeds compared to a major worker not specialized to mill. Therefore seed-harvesting majors should have musculature optimized for force production and correspondingly wider heads, relative to head length. Finally, because a switch in diet to seed-harvesting is considered adaptive in habitats where food availability is unpredictable (Wheeler, 1910), we hypothesized that seed-harvesting lineages will display faster rates of evolutionary change and increased diversification permitted by leveraging a new and abundant food source.



**Figure 1.** *Pheidole metallescens*, a non-seed-harvesting species, major worker (left) and minor worker (right). (c) 2013, Alexander Wild, reused with permission.

## MATERIAL AND METHODS

### SPECIES SELECTION, SEED-HARVESTING, AND MORPHOMETRICS

We mapped head width and head length measurements of majors and minors onto a phylogeny for 60 species of *Pheidole* and examined patterns of change related to foraging preferences. Species were chosen because they met three criteria: (1) they were included in the phylogeny of Moreau (2008) and so their evolutionary relationships to other species were known; (2) data were available on dietary preferences (seed-harvesting presence/absence); and (3) morphometric data were available from Wilson's (2003) revision of New World *Pheidole*. Of the 60 species included in our analyses, 31 species are primarily seed-harvesters and the other 29 species are insectivorous or generally omnivorous. We excluded the two species with a trimorphic worker because the presence of a super-major may influence the

morphology of majors and minors in ways unrelated to diet. Seed-harvesting data were taken from Moreau (2008), by S. P. Cover (pers. comm.), Hölldobler & Wilson (1990), Johnson (2000), and Wilson (2003).

We used three linear morphometric measurements: two that provide information on head size and shape [head width (HW) and head length (HL)], as well as pronotal width (PW), which is the best single predictor of total mass for ants in the subfamily Myrmicinae ( $r^2 = 0.96$ ; Kaspari & Weiser, 1999). HW, HL, and PW measurements were log-transformed for all analyses. The difference between major and minor HW and HL (DiffHW, DiffHL) was calculated by subtracting the minor head size from the major head size and log-transforming the result. We also calculated a head shape index by dividing untransformed HL by HW. Ants with a head shape index of 0 have square-shaped heads,  $< 0$  indicates heads that are longer than broad, and  $> 0$  indicates ant heads that are broader than long.

#### PHYLOGENY

The phylogenetic tree used in the comparative analyses is a maximum likelihood chronogram adapted from Moreau (2008). The molecular phylogeny was inferred in GARLI, version 0.94 (Zwickl, 2006). This tree pruned of all species without seed-harvesting data using MESQUITE, version 2.75 (Madison & Madison, 2011). This pruned maximum likelihood chronogram contains 60 species.

#### STATISTICAL ANALYSIS

We hypothesized that the major subcaste of seed-harvesting *Pheidole* species will have wider heads than generalist species and the difference in head size between subcastes would be greater in seed-harvesting species. Associations between HL and HW for each subcaste were examined with allometric analysis implemented in R, version 2.15.2 (R Development Core Team) using the standardized major axis estimation function from the *smatr* package (Falster *et al.*, 2006; Warton *et al.*, 2006). We also investigated the relationship between PW and seed-harvesting. If head size variation between seed-harvesters and non-seed-harvesters is representative of overall body size, then PW variation should display the same patterns as HW and HL for majors, minors, and the difference between majors and minors. If, however, the patterns of PW variation differ from HW and HL then it is likely that head morphology is evolving relative to a selective pressure (such as diet) independent of overall body size. Therefore, we also compared regression slopes of PW to majors and minor HW and HL, and between seed-harvesting and non-seed-harvesting species.

To further test whether differences in head size are associated with seed-harvesting, we calculated phylogenetic independent contrasts (PICs) (Felsenstein, 1985; Harvey & Pagel, 1991). This aimed to compare differences in head shape between the two diet groups, at the same time as accounting for the phylogenetic relationships between the species (which is not accounted for in the standardized major axis estimation). In all cases, HL, HW, and the head shape index were compared with respect to whether species do (or do not) harvest seeds. These data met the requirements of the PICs; the Brownian motion model of evolution best fits the data and the contrasts were standardized using the branch length method of Nee (Garland *et al.*, 1992; Purvis, 1995). This was a conservative transformation; no PICs produced unique statistically significant results with this branch transformation, and the results were replicated using alternative branch transformations (not shown). We hypothesized that a change in diet is associated with a larger change in HW, HL, Diff HW, and Diff HL. Because the contrast data violated requirements for parametric tests, we used a Mann–Whitney *U*-test to compare the contrasts where there was a switch in diets to contrasts when species did not switch diets to test this hypothesis.

To conduct the analyses in a phylogenetic context, we first tested that the head size measurements of closely related species were morphologically more similar to each other. This was carried out using Pagel's lambda (Pagel, 1999) to infer the amount of phylogenetic signal in all head size characters. If there is phylogenetic signal on these characters, then a change in head size associated with diet is an indication of selection acting on head size. If there is no phylogenetic signal, then changes in head size cannot be attributed to adaptation to diet. The lambda parameter ( $\lambda$ ) ranges from 0 to 1, with values close to 0 representing no phylogenetic signal of trait distributions and increasing phylogenetic signal in traits as  $\lambda$  approaches 1. We performed three  $\lambda$  transformations of the phylogenetic tree ( $\lambda = 0, 0.5, 1$ ) and then fit the character data to the three trees. The best model was inferred by a difference in corrected Akaike information criterion of 4 or more (Burnham & Anderson, 2002), and is demonstrated by the highest Akaike weight of the three tests. The evolution of head size was explored by modelling rate shifts across the phylogeny using all six measurements for head size (major and minor HW, major and minor HL, Diff HW, and Diff HL). This hypothesis was modelled using the *transformPhylo.ML* function of the R package *MotMot* (Thomas & Freckleton, 2011). We analyzed the data with two rate shift models (tm1 and tm2), and both identified eight identical relative rate estimates. All statistical

analyses were performed using R, version 2.15.2 (R Development Core Team), with the exception of the PICs and branch transformations, which were conducted in MESQUITE, version 2.75 (Madison & Madison, 2011).

## RESULTS

### STANDARDIZED MAJOR AXIS ESTIMATION

We used standardized major axis estimation to determine whether head width and length differed between seed-harvesting species and non-harvesting species (Fig. 2; measurements in Table 1). The head width and head length of majors showed tight allometric relationships for both seed-harvesters ( $r^2 = 0.952$ ,  $P < 0.0001$ ) and non-harvesters ( $r^2 = 0.954$ ,  $P < 0.0001$ ) and there was no difference between the regression slopes of these groups based on diet (Fig. 2A, SAM, likelihood ratio statistic = 0.0143, d.f. = 1,  $P = 0.905$ ). Minor head width and head length also had strong allometric relationships for seed-harvesters ( $r^2 = 0.861$ ,  $P < 0.0001$ ) and non-harvesters ( $r^2 = 0.7995$ ,  $P < 0.0001$ ), although less variation was explained by the model. There was a difference between the regression slopes for minor subcaste head characters based on diet (Fig. 2B, SAM, likelihood ratio statistic = 4.027, d.f. = 1,  $P = 0.0448$ ); the head length of minor workers from seed-harvesting species did not increase at the same rate as the head length of non-harvesters.

We investigated pronotal width, a correlate of body mass, to identify whether differences in head size reflect changes in overall body size. In all regression analyses, there was no difference between seed-har-

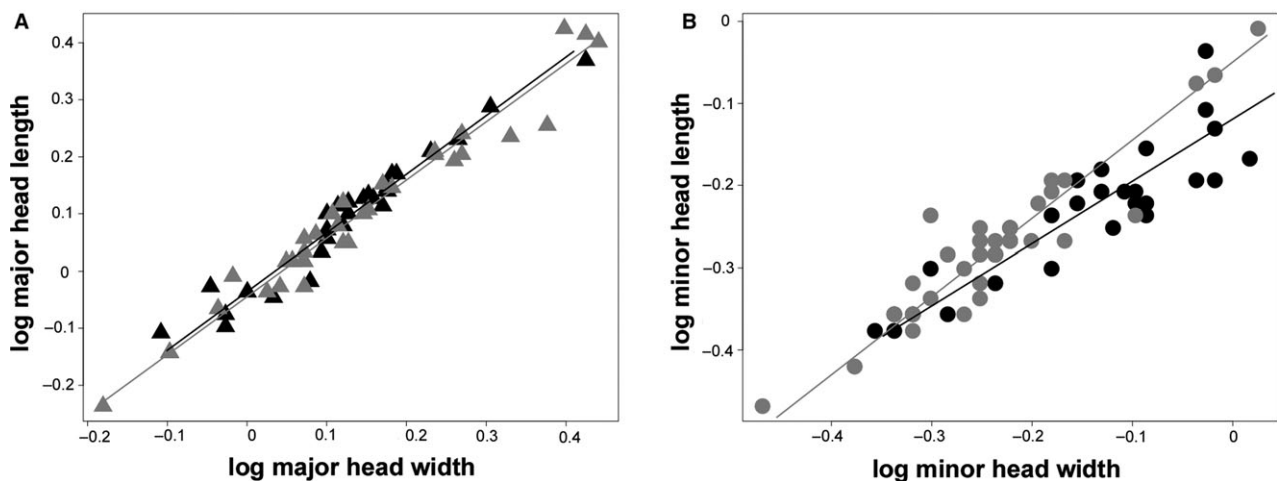
vesters and non-harvesters when comparing pronotal width with head length and width of majors and minors (Fig. 3, Table 2). Therefore, the differences in minor head length based on diet are not simply a reflection of changes in overall worker body size.

### PICs

We conducted PICs to determine whether head size diverged more greatly between related species that exhibited a diet shift to seed-harvesting. There was no significant relationship between head size and diet for HW or HL for either caste (majorHW  $r^2 = 0.0151$ ,  $P = 0.342$ ; majorHL:  $r^2 = 0.042$ ,  $P = 0.111$ ; minorHW  $r^2 = 0.0128$ ,  $P = 0.931$ ; minorHL  $r^2 = 0.0011$ ,  $P = 0.794$ ) (Fig. 4), indicating that related species do not undergo a rapid change in head morphology with a diet shift. By contrast, the differences between major and minor head width and head length did show a relationship with change in diet (DiffHW  $r^2 = 0.0139$ ,  $P = 0.013$ ; DiffHL  $r^2 = 0.0858$ ,  $P = 0.021$ ).

We conducted Mann–Whitney *U*-tests to determine whether independent contrasts between related species that had undergone a diet switch statistically differed from related species that shared diet preferences. These results supported the PICs (Table 3). A change in diet is associated with a larger difference in head size between castes, although major and minor head size individually did not change size when species change diets.

We also predicted that seed-harvesters should have broader heads, as manifested by a size index  $> 1$ . A PIC of majors revealed no relationship between head shape and diet ( $r^2 = 0.0021$ ,  $P = 0.72$ )



**Figure 2.** Standardized major axis estimation regressions of worker head width and head length and length for majors (triangles) and minors (circles). A, major head width vs. major head length. B, minor head width vs. minor head length. Grey markers indicate seed-harvesting species; black markers indicate non-seed-harvesting species.

**Table 1.** Mean head size (mm)  $\pm$  standard error of the mean (SEM) for each species of *Pheidole* based on diet. Head measurements for each species were log transformed prior to calculating the mean

	Seed-harvesters		Non-harvesters	
	Mean $\pm$ SEM (mm)	Log mean $\pm$ SEM (mm)	Mean $\pm$ SEM (mm)	Log mean $\pm$ SEM (mm)
Major HW	1.33 $\pm$ 0.12	0.09 $\pm$ 0.03	1.25 $\pm$ 0.07	0.08 $\pm$ 0.02
Major HL	1.65 $\pm$ 0.19	0.15 $\pm$ 0.02	1.35 $\pm$ 0.07	0.12 $\pm$ 0.02
Minor HW	0.51 $\pm$ 0.03	-0.27 $\pm$ 0.02	0.59 $\pm$ 0.02	-0.24 $\pm$ 0.02
Minor HL	0.55 $\pm$ 0.03	-0.24 $\pm$ 0.02	0.72 $\pm$ 0.03	-0.16 $\pm$ 0.02
Diff HW	0.82 $\pm$ 0.09	-0.13 $\pm$ 0.03	0.67 $\pm$ 0.05	-0.21 $\pm$ 0.03
Diff HL	1.1 $\pm$ 0.15	-0.09 $\pm$ 0.03	0.63 $\pm$ 0.05	-0.23 $\pm$ 0.03
Major PW	0.64 $\pm$ 0.05	-0.21 $\pm$ 0.02	0.64 $\pm$ 0.03	-0.21 $\pm$ 0.02
Minor PW	0.33 $\pm$ 0.02	-0.47 $\pm$ 0.02	0.40 $\pm$ 0.02	-0.41 $\pm$ 0.02
Diff PW	0.31 $\pm$ 0.03	-0.58 $\pm$ 0.04	0.24 $\pm$ 0.02	-0.66 $\pm$ 0.03
Major head Shape index	1.10 $\pm$ 0.01		1.08 $\pm$ 0.01	
Minor head Shape index	1.10 $\pm$ 0.02		1.21 $\pm$ 0.03	

HW, head width; HL, head length; PW, pronotal width.

and the same applied to minor worker head shape ( $r^2 = 0.0344$ ,  $P = 0.15$ ) (see Supporting information, Fig. S1).

#### HEAD SIZE RATE SHIFTS

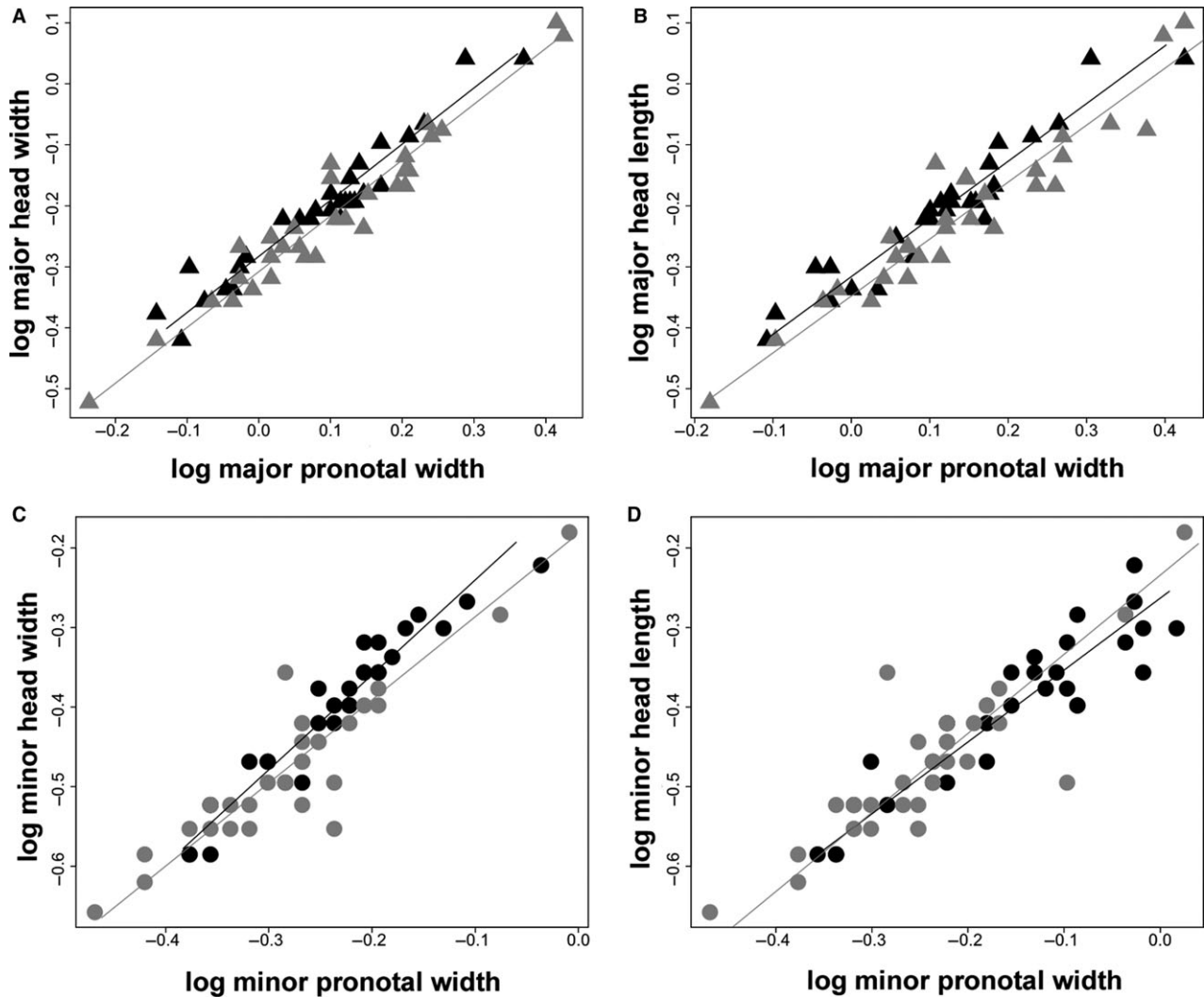
All *Pheidole* head size characters best fit the  $\lambda = 1$  transformed tree (see Supporting information, Table S1). This indicates that the inferred phylogeny predicts trait distributions and there is strong phylogenetic signal in the head morphology. The phylogeny was used to estimate evolutionary rate shifts (Fig. 5), and all rate shifts were faster than the modelled background rate of change, with one exception, *Pheidole nitella* (node 6, ML rate =  $1 \times 10^{-8}$ ). Node 1 therefore indicates a rate shift at a node including almost all taxa, which is likely to account for the slow rate of change of *P. nitella*. Several species showed a slower rate of change than most species (but faster than the baseline rate of change): the *Pheidole tristicula* and *Pheidole rhinoceros* clade (node 3, ML rate = 10.76). Two individual species showed accelerated rates of change: the seed-harvester *Pheidole pilifera* (node 5, ML rate = 1000) and the non-seed-harvesting *Pheidole astur* (node 2, ML rate = 1000), and two clades stand out as evolving faster. These are denoted by node 4 (ML rate = 558.9), a group of seed-harvesters and non-harvesters, and node 7, a clade of three desert seed-harvesters (ML rate = 762.6). The clade indicated at node 7 includes *Pheidole barbata*, *Pheidole militica*, and *Pheidole psamophilla*, all possessing workers with a particularly large difference in the head size of majors and minors. We repeated the PICs without this clade and obtained the same results (not presented), and so this clade contributes to our

main finding but does not drive it. There was no overall relationship between diet and rate of evolution; rate shifts did not occur as *Pheidole* species switched to or from seed-harvesting.

#### DISCUSSION

Understanding how size and shape evolves among related taxa, and how those changes may be driven by foraging ecology, has been a longstanding goal of evolutionary ecologists (Wainwright & Richard, 1995; Wainwright & Reilly, 1994; Grant, 1999; Richmond & Price, 2002; Dumont *et al.*, 2012). Eusocial insects are a particularly interesting group for such analysis because individuals in the group are divided into morphologically distinct castes. In approximately 13% of ant species, workers are further subdivided into morphologically variable subcastes (Wilson, 1953; Oster & Wilson, 1978), although this is rare among other eusocial Hymenoptera. The evolution of distinct subcastes allows form and function to diverge among sisters in the same colony (Powell, 2009).

We mapped characters related to head size, head shape, pronotal width (a surrogate for body size), and diet onto a phylogeny of the dimorphic ant genus *Pheidole* aiming to understand how shape evolves in relation to specialization for seed-harvesting in a genus with two distinct worker castes. We found that head width and length of major and minor workers show a strong phylogenetic signal, and that the difference in head size between the subcastes is related to diet. Specifically, seed-harvesting species have a more pronounced size difference in head size between the major and minor workers. By contrast



**Figure 3.** Standardized major axis estimation regressions of worker pronotal width and head width and length for majors (triangles) and minors (circles). A, pronotal width vs. major head width. B, pronotal width vs. major head length. C, pronotal width vs. minor head width. D, pronotal width vs. minor head length. Grey markers indicate seed-harvesting species; black markers indicate non-seed-harvesting species.

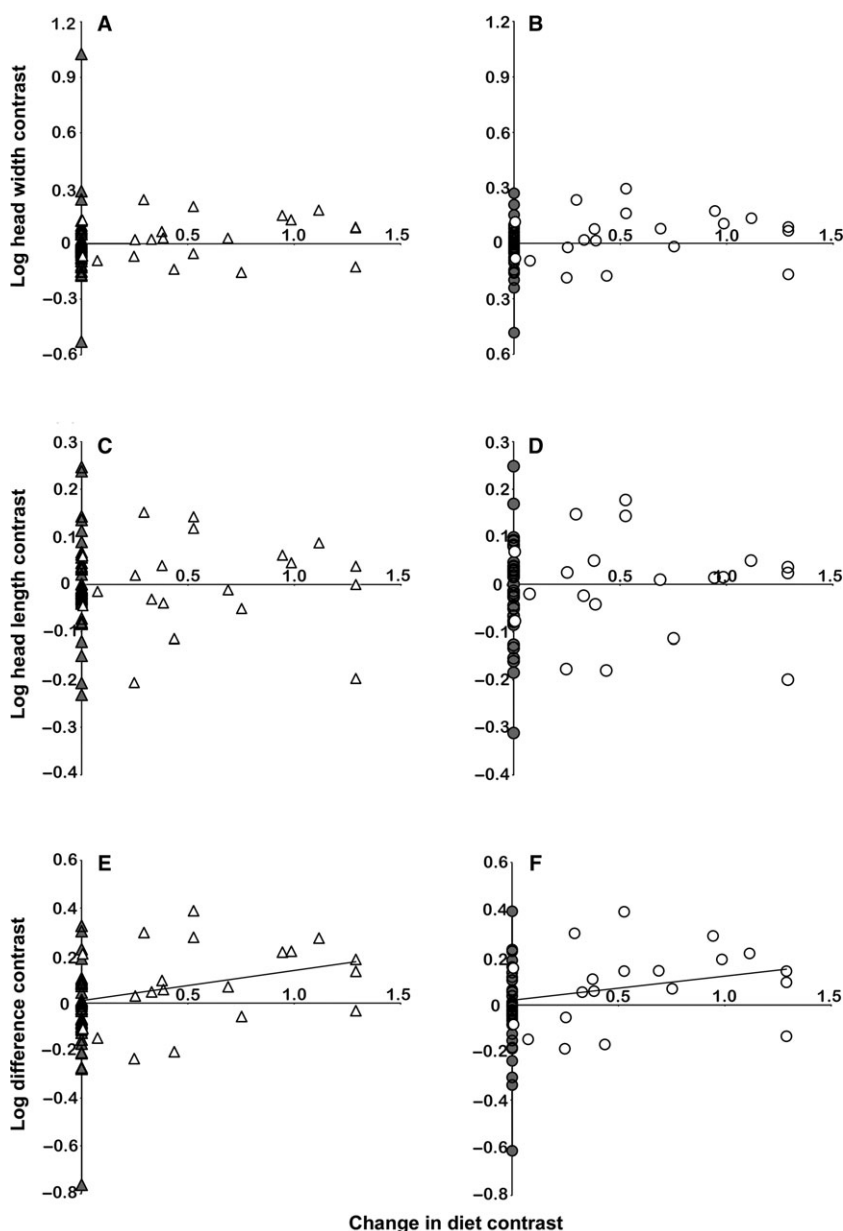
**Table 2.** Results of the standardized major axis estimations of pronotal width regressed against head width and length for majors and minors (Fig. S1). The fit of the regressions for head size variables are listed for seed harvesters and non-harvesters separately

Head character	Seed-harvesters ( $r^2$ )	Non-harvesters ( $r^2$ )	Log-likelihood ratio	$P$ -value
Major HW	0.935	0.933	<b>0.0078</b>	0.929
Major HL	0.907	0.917	<b>0.027</b>	0.87
Minor HW	0.832	0.927	1.984	0.159
Minor HL	0.743	0.853	0.6237	0.4297

HW, head width; HL, head length; PW, pronotal width. Significant values are bolded.

to our predictions, head sizes of majors did not increase in seed-harvesting lineages, and minor workers of seed-harvesters have smaller, wider heads than minors of non-seed-harvesters. However, this result was only observed when the phylogenetic relationships were not accounted for, indicating that wide heads on seed-harvesting minors may be restricted to a group of related species. We also examined whether related species that differed in diet had a larger difference in head-size compared to related species that do not differ in dietary habits. We did not see a positive slope in the PICs, although there was a positive relationship between diet shifts and the difference in head size between castes.

We expected seed-harvesting species to have majors with wider heads than non-seed-harvesting



**Figure 4.** Phylogenetic independent contrast (PIC) regressions of worker head size and diet (seed-harvesting or not). When change in diet is  $> 0$ , this indicates a switch to or from seed-harvesting; when 0, this indicates related species remained as seed-harvesters or non-seed-harvesters. A, major head width. B, minor head width. C, major head length. D, minor head length. E, the difference between major and minor head width. F, the difference between major and minor head length. White markers indicate contrasts where related species changed diet; gray markers indicate no diet change. Triangles represent head width contrasts; circles represent head length contrasts.

species. This prediction arose from several observations. First, majors have been reported to be a specialized seed-milling caste in some *Pheidole* (Wheeler, 1910). Second, mandibular force production is controlled by parallel muscle fibres attached to the apodeme by filaments in the head capsule, and is correlated with the broadness of the head (Paul & Gronenberg, 1999). Finally, head size mor-

phology varies between major and minor *Pheidole* workers, whereas other characters appear to be constrained (Pie & Traniello, 2007). We predicted that the differences between seed-harvesting and non-harvesting majors would be sufficient to produce larger mandible closer muscles and wider heads, despite constraints on morphological evolution in subcastes (Pie & Traniello, 2007). By contrast,



**Table 3.** Results of the Mann-Whitney U test comparing independent contrasts of related species that do have a diet switch compared to those that do not have a diet switch (data from Fig. 4)

Head size character	Mann-Whitney <i>U</i> -value	<i>P</i> -value
Major HW	515.5	0.105
Major HL	536	0.0533
Minor HW	374	0.5802
Minor HL	371	0.549
Diff HW	259	0.0199*
Diff HL	272	0.0339*

HW, head width; HL, head length; PW, pronotal width. \* denote a significant difference between the two diet groups.

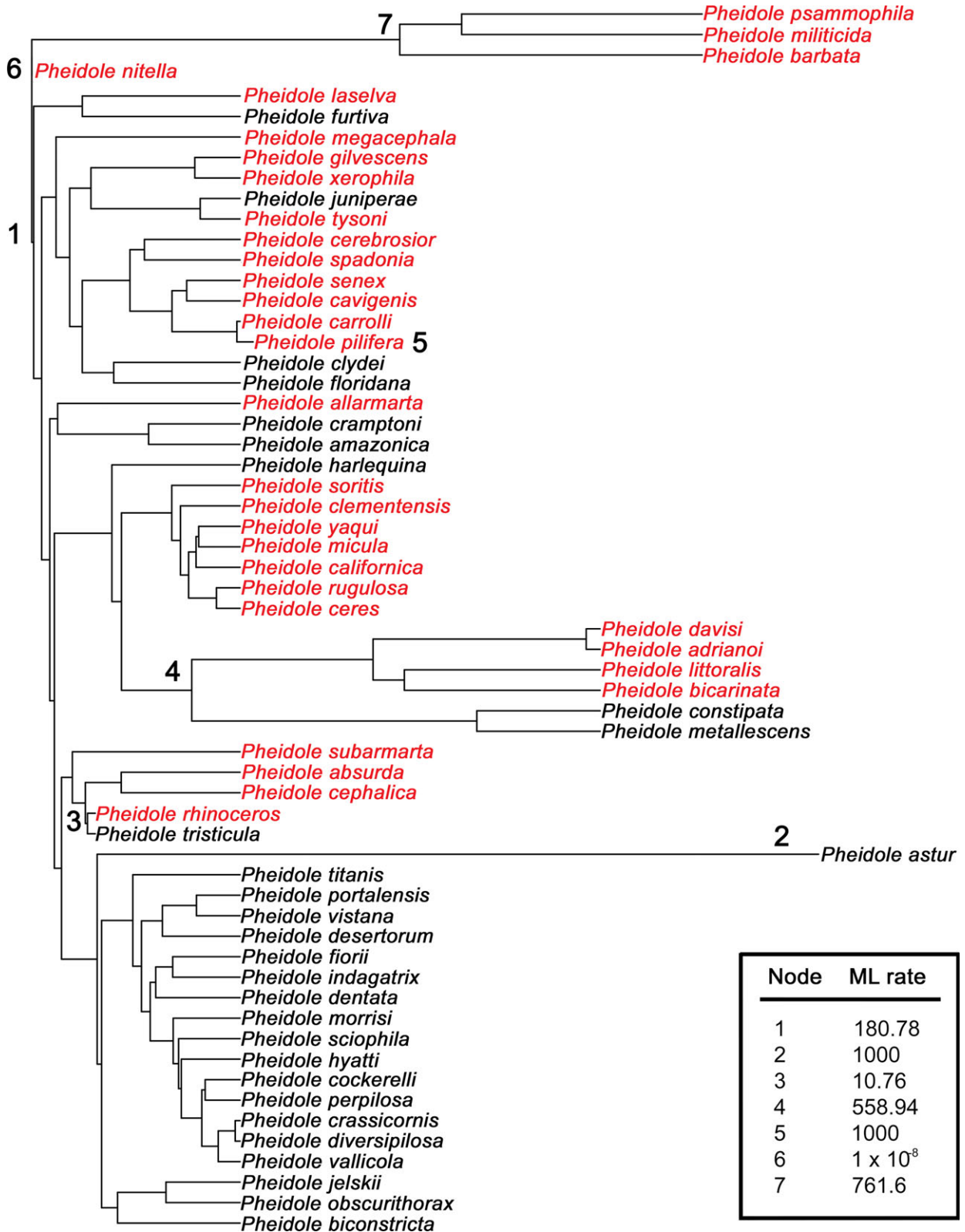
Wheeler (1910) argued that the head morphology of seed-harvesting ants is similar to insectivorous species because the musculature required for crushing seeds was also needed for carving up insect exoskeletons. The finding of no relationship between head shape of majors and seed-harvesting (Figs 2, 3) concurs with the conclusions of Wheeler (1910) and Pie & Traniello (2007). Pie & Traniello (2007) found that worker morphology varies predominantly in size alone, even though *Pheidole* inhabit broad ecological niches. Our finding thus supports their conclusion that size, represented by differences between majors and minors, is the primary way species vary morphologically, not shape. We investigated head width and length, however, and so it remains possible that differences between seed-harvesters and generalists lay in other properties such as mandible size and shape.

Further research is required not only on both the head morphology of *Pheidole*, but also their seed processing behaviour. The present study was limited to two characters, head length and width, and so a more detailed examination of head shape may reveal that seed-milling majors have a different head shape compared to related non-milling species in more subtle ways (Ferster *et al.*, 2006). The unrelated seed-harvesting ants *Pogonomyrmex badius* and *Solenopsis geminata* both have larger occipital regions of the head (above the eyes), and larger head width, when compared allometrically with seven other head and body measurements (Ferster *et al.*, 2006; Tschinkel, 2013). Additionally, the seed-harvesting/non-harvesting dichotomy used in the present study is likely oversimplified. *Pheidole* species may put seeds to different uses; perhaps not all supposed 'seed-harvesting' *Pheidole* majors are milling seeds; instead, workers may collect them for the lipid-rich eliaosomes exploited by many ant species (Sernander, 1906). Seed-harvesting species, however, are concentrated in regions without rich diversity of eliaosome producing trees (Lengyel

*et al.*, 2009), suggesting the eliaosomes are not driving seed collection (Beattie, 1985), although it not known how *Pheidole* process the eliaosome-bearing seeds they collect (Hughes, Westoby & Jurado, 1994; Cuautle, Rico-Gray & Diaz-Castelazo, 2005). Thus, further studies on seed use in *Pheidole* will require direct observation of seed collection and milling.

The difference in head size between major and minor workers was greater in seed-harvesting species of *Pheidole* (Figs 2, 4). Dimorphic worker subcastes have been associated with a dietary switch to seed-harvesting in *P. badius* and *S. geminata* because their non-harvesting relatives are monomorphic (Ferster *et al.*, 2006). In these species, the major subcaste specializes on seed-milling to supplement other food sources collected by the minor workers (Hölldobler & Wilson, 1990; Ferster *et al.*, 2006). There was also a faster relative rate of head size evolution in a clade of seed-harvesting, desert-dwelling *Pheidole*, all of which possess particularly large majors and small minors (Fig. 4: clade 6: *P. barbata*, *P. militica*, and *P. psammophila*). These findings may indicate a more pronounced division of labour between seed-milling majors and minors. An alternative explanation for our findings is that selection is acting on the size of minors as a result of their role in territorial defence (Langen, Tripet & Nonacs, 2000), or that nesting habitat, which coincides with diet, drives head size differences (i.e. seed-harvesting occurs among soil-living species). Twig-nesting and canopy species may have difficulty producing dry chambers to store seeds. A large difference in body size between majors and minors was also observed in ground dwelling *Pheidole* species in the tropics (Mertl *et al.*, 2010), which was suggested to be associated with defence.

Among the ants, several genera display subcaste variation as an adaptation to food processing. Attine leaf-cutting ants have continuously polymorphic workers that cut or masticate leaf fragments based on size (Wilson, 1980). Powell & Franks (2006) demonstrated that the submajor worker subcaste was present in three army ant species known to handle 'awkwardly shaped' prey. Dimorphic worker subcastes are present in seven ant genera (Oster & Wilson, 1978) and are often associated with nest defence (e.g. *Colobopsis* and *Cephalotes*; Powell, 2008). Using a phylogeny spanning almost all ant genera (Moreau *et al.*, 2006), Pie & Tschá (2013) found that size evolves much more quickly than shape among ant genera. By contrast to our findings, within the genus *Pheidole*, they concluded that there was a decelerating rate of change in body size in major workers of the seed-harvesting desert-dwelling clade (Pie & Tschá, 2013). We found that relative rate of head size change was faster than the baseline rate of change in all taxa but one (*P. nitella*).



**Figure 5.** Head size rate shifts in *Pheidole*. Branch lengths are scaled to the maximum likelihood relative rate estimates for change in head size (rates listed in the inset table). Node numbers denote where the seven rate shifts were observed. Red names indicate seed-harvesting species.

The rate of head size evolution was not associated with diet; head size of seed-harvesting species had the same estimated rate of change as non-harvesting species in most cases. This suggests that diet switching does not lead to an increased rate of morphological change and refutes the hypothesis that switching to seed-harvesting allowed *Pheidole* species to diversify rapidly with access to a new food source. Pie & Traniello (2007) found that morphological variation among *Pheidole* species was driven largely by differences in size (size explained up to 83% and 78% of the variation among majors and minors, respectively, in a principal component analysis) and not by large changes in shape. Within species, the strongest differences between castes were associated with variation in head width, head length, and thorax length (Pie & Traniello, 2007). A recent study of global *Pheidole* demonstrated that body size variation was similar in Old and New World species, and that *Pheidole* communities repeatedly evolve similar size distributions despite inhabiting a range of biomes (Economo *et al.*, 2015). These results also imply strong constraints on body size evolution. Future work at community scales is still needed to determine whether *Pheidole* species assemblages show divergence in morphology with respect to diet partitioning (e.g. to escape competition).

The production of the major subcaste results from differences in larval nutrition and juvenile hormone levels during development (Wheeler & Nijhout, 1981; Wheeler, 1986). Moreover, the ability to produce ‘supermajors’, a third worker subcaste (Huang, 2012), appears to be a conserved trait among *Pheidole* species, although it is rarely expressed (Rajakumar *et al.*, 2012). Supermajor development can be elicited in majors with the application of a juvenile hormone analogue at the appropriate developmental window (Rajakumar *et al.*, 2012). Taking an Eco-Evo-Devo approach (Fave *et al.*, 2015) combining an examination of the developmental basis of traits with an understanding of the ecological factors and why they arise will be paramount for understanding the evolutionary basis of worker variation in ants. Moreover, examining polymorphism in morphology in castes other than workers [e.g. queens (Bespalova & Helms, 2014; Fave *et al.*, 2015) and males (Heinze & Hölldobler, 1993)] will provide further insight into its origin and function.

The reason for the hyperdiversity of *Pheidole* is unknown, although Wilson (2003) suggested that it may result from having a dimorphic worker subcaste. In particular, the ubiquitous big-headed major allowed *Pheidole* species to become ecologically dominant; their head morphology enables species to fill a variety of specialized roles such as defence, guarding, and food-gathering, whereas the minor caste retains a gen-

eralized function, resisting morphological change (Wilson, 2003; Pie & Traniello, 2007; Mertl & Traniello, 2009; Mertl *et al.*, 2010). Alternately, *Pheidole* may have adapted behaviourally rather than morphologically, leading to niche differentiation and subsequent speciation with little morphological change (Mertl *et al.*, 2010). Furthermore, the proportion of major workers to minors varies from species to species, seasonally, and in response to resource availability, adding yet another means of restructuring the worker force (McGlynn *et al.*, 2012). Essentially, *Pheidole* may meet new ecological challenges by drawing from existing castes or altering their relative representation. These characteristics allow the genus to maintain a flexible worker force that can adjust to different habitats and food sources quickly, without relying on conspicuous morphological evolution. These results indicate that *Pheidole* morphology evolves more readily by size but not shape. It also highlights the importance of size differences between worker subcastes, which may vary according to diet and potentially other factors. Although this does not explain *Pheidole*'s hyperdiversity, it lends further support for why a major subcaste may contribute to the ecological success of this genus.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Phylogenetic independent contrast (PIC) regressions of worker head shape and diet (seed-harvesting or not).

**Table S1.** Fit of lambda ( $\lambda$ ) transformed phylogenetic trees to *Pheidole* head size characters and the difference between majors and minors (Pagel, 1999).