ORIGINAL RESEARCH

Size and shape in the evolution of the worker head in *Pheidole* ants (Hymenoptera: Formicidae)

A. Casadei-Ferreira[1,2] (iD), R. M. Feitosa[1] (iD) & M. R. Pie[1,3] (iD)

[1]Departamento de Zoologia, Setor de Ciências Biológicas, Centro Politécnico, Universidade Federal do Paraná, Curitiba, Paraná, Brazil
[2]Biodiversity and Biocomplexity Unit, Okinawa Institute of Science and Technology Graduate University, Onna, Okinawa, Japan
[3]Biology Department, Edge Hill University, Ormskirk, Lancashire, UK

Abstract

The division of labor into sterile and reproductive castes in social insects is often reflected in marked morphological differences, which might have played an important role in the remarkable adaptive success of these organisms. Some ant lineages have undergone further morphological differentiation, with the evolution of differences within the worker caste. In this study, we characterize morphological diversity in the head of *Pheidole* ants by comparing differences in size and shape among species and between minor and major worker subcastes. To this end, we integrate data from high-resolution images, geometric morphometrics, and phylogenetic comparative methods. Our results indicated differences in morphological variation of each subcaste with respect to their geographical distribution, with distinct morphological patterns and evolutionary routes related to head shape. Allometry was shown to be a crucial element for the differentiation within and between each subcaste, corroborating the role of size in their morphological evolution. Additionally, we observed that closely related species often diverge considerably in morphospace, whereas convergence in their morphospace occupation characterizes some West and East Hemisphere species. Finally, although multiple shifts in the rate of morphological evolution occurred during the Miocene, the timing and position of these shifts were independent of size and shape, suggesting that their evolution has been decoupled throughout *Pheidole* evolution.

Introduction

Colonies of social insects are often considered superorganisms due to the existence of a self-organized workforce characterized by efficient and cohesive communication systems (Hölldobler & Wilson, 2009). Ant workers are frequently specialized in a subset of tasks necessary to maintain the colony (Wheeler, 1911). In some ant lineages, task specialization can be partially achieved through anatomical modifications, also known as caste polymorphism (Molet et al., 2012; Trible & Kronauer, 2017) or physical castes (Oster & Wilson, 1978), in which these modifications were attained through the worker's potential to produce a wide variety of new subcastes. These novel morphologies can emerge from different mechanisms, with individuals occupying new positions in phenotypic space through distinct developmental routes (Trible & Kronauer, 2017).

Among ants, the hyperdiverse and cosmopolitan genus *Pheidole* is well-known for its conspicuously dimorphic workers (Molet et al., 2012; Oster & Wilson, 1978; Traniello, 2010), which include more slender forms referred to as minors and

robust conditions known as majors (also known as soldiers; Oster & Wilson, 1978; Traniello, 2010; Wilson, 2003). The presence of majors has been considered the key to the remarkable success of *Pheidole* (Traniello, 2010; Wilson, 2003). Majors defend the colony, occupying the nest galleries and chambers, are recruited by minors to carry out functions outside the nest (e.g., transporting food), and are responsible for food processing (Moreau, 2008; Wilson, 2003). This set of morphological and behavioral adaptations is strongly variable and is considered crucial in enhancing colony survival due to worker specialization in specific tasks (Oster & Wilson, 1978; Traniello, 2010; Wilson, 2003).

Even with the substantial morphological differences among *Pheidole* species, most of these differences can be attributed to allometry coupled with a low degree of morphological integration within its subcastes (Friedman et al., 2019, 2020; Pie & Traniello, 2007). Holley et al. (2016) also demonstrated a considerable difference in head size between granivorous and non-granivorous *Pheidole* species. However, little has been explored concerning these morphological variations in a phylogenetic

context (e.g., Friedman et al., 2019, 2020; Pie & Traniello, 2007; Pie & Tschá, 2013).

This study aims to describe the variation in head shape in the worker caste of *Pheidole*, focusing on exploring patterns related to its geographical distribution and evolutionary processes that may have led to their current morphological diversity. Insects have an extraordinary variety of exaggerated morphologies (Emlen & Nijhout, 2000) that can be sexually (e.g., enlarged horns in beetles; Arrow, 1951) and non-sexually (e.g., soldiers in eusocial insects; Oster & Wilson, 1978) selected, making them an ideal group to explore the variation in shape and evolutionary trends of such traits. Therefore, describing how different subcastes of eusocial organisms are distributed across morphological space, quantifying the size-dependent and size-independent variation in their morphology, and exploring the evolution of their traits can illuminate our understanding of how exaggerated structures evolve in dimorphic groups, especially in hyperdiverse lineages such as *Pheidole*.

Our goals were (1) to describe the morphospace occupied by majors and minors; (2) to assess the geographic patterns of morphological evolution for each subcaste; and (3) to estimate the rate shifts of shape evolution and the morphospace occupation of majors and minors in their evolutionary history and distribution.

We test three predictions. First, we expect that different subcastes and species would occupy distinct regions of morphospace, even if the size effect is removed. Oster and Wilson (1978) have proposed that the caste distribution in a given lineage evolves to match the resource distribution, which would mean that novel caste morphotypes are expected to represent the evolution of novel functional interactions with a resource base. In this scenario, ant lineages that present such morphological adaptation in their castes are thought to show increased ergonomic optimization for the colony, such that caste evolution provides functionally improved performance in colonial tasks by morphological specialization (Mertl & Traniello, 2009; Oster & Wilson, 1978; Powell, 2016). Interspecific differences in the morphology of workers' subcastes can be substantial since the adaptive radiation in lineages with such division are often successful in terms of numbers of species and environmental dominance and occupancy (Moreau, 2008; Pie & Traniello, 2007; Powell, 2016; Powell et al., 2020). We expect major workers to display distinct and size-independent shape patterns from minors due to their specialization in particular functional roles (Friedman et al., 2020; Oster & Wilson, 1978; Powell, 2016; Trible & Kronauer, 2017).

Second, we predict that closely related lineages experience morphological divergence, whereas convergence is expected for distant lineages. The colonization of novel environments might result in adaptive divergence (Friedman et al., 2020; Liu et al., 2020; Parsons & Robinson, 2006; Powell, 2016, Powell et al., 2020), reflecting in selecting a new optimal phenotype state that positively affects colony ergonomic efficiency. Thus, this process will create consistent patterns of phenotypic similarity repeatedly and independently from each colonization in similar habitats. Powell et al. (2020) have demonstrated this pattern in the ant genus *Cephalotes*, in which the four soldiers morphotypes (based on the head shape) repeatedly evolved, with consistent gains and losses, through its evolution.

Finally, we expect an acceleration in the rate of major and minor head shape diversification coinciding with *Pheidole* adaptive radiation and, consequently, niche filling dynamics, while decelerations and periods of morphological constraint will tend to be less frequent (see Harmon et al., 2010). Economo et al. (2019) demonstrated that *Pheidole* probably experienced three main shifts in its diversification, with an initial shift approximately 15–16 Ma in the New World; a second with the colonization of the Old World (~13 Ma); and a third in the colonization of the Australia-New Guinea (~9 Ma). Additionally, these authors showed evidence that ecological opportunity somewhat controls the diversification rate in *Pheidole*, in the process of radiation through an initial acceleration with new colonization, followed by a deceleration attributed to the niche filling.

A high frequency of positive shifts in morphological diversification, compared to negative shifts, has already been observed in *Pheidole* using linear head measurements (see Holley et al., 2016). Contrary to this pattern, Pie and Tschá (2013), employing similar approaches as Holley et al., demonstrated that large-scale shifts in several ant lineages are associated with decreases in evolution rates. Pie and Tschá also showed that most negative shifts are associated with size (PC1; Fig. 3, pg. 9) in *Pheidole* majors, while in minors, these negative shifts are related to shape (PC2; Fig. 4, pg. 10). We hypothesize that with the availability of novel niches in colonization events, through an accelerated filling of these niches, *Pheidole* experienced accelerations in its head shape diversification that coincide with shifts evidenced by Economo et al. (2019) with few decelerations during this process.

Materials and methods

Data acquisition

We compiled a database of *Pheidole* major and minor worker images (one specimen per subcaste) from AntWeb.org (Antweb, 2019; available at http://antweb.org, last accessed in 2019) based on the species included in the phylogeny by Economo et al. (2015). Both West Hemisphere (WH) and East Hemisphere (EH) species were included to maximize the geographical and phylogenetic breadth of the study, using the Atlantic Ocean as a criterion for this separation. We assumed that intraspecific variation is relatively small compared to interspecific variation in the genus. We tried to select specimen photos whose position in the entomological pin/triangle allowed for reliable landmark acquisition. All used images included their corresponding scales (from 0.2 to 1.0 mm). In total, our database included 168 major worker images (79 WE and 89 EH; $N = 168$ specimens) and 178 minor worker images (65 WE and 113 EH; $N = 178$ specimens; Table S1), which represents, respectively, ~16 and ~17% of the currently recognized *Pheidole* species (Bolton, 2021). These species also represent ~58 and ~62% of the 285 taxa included in the reference phylogeny, respectively. The combined database, consisting of those species for which we had images of both subcastes, encompasses 112 species (61 WE and 61 EH), accounting for

~11% of the genus species richness and ~39% of the phylogeny tips.

Geometric morphometrics

Head shape was measured by digitizing two landmarks and 20 semilandmarks using the tpsDig v. 2.31 (Rohlf, 2021). We chose to use this selection of anatomical semi- and landmarks due to our focus on describing changes related to the head shape pattern while not including variation in other cephalic structures that could potentially be used as landmarks (i.e., antennal insertion, eye position, frontal triangle, etc.). The landmarks corresponded to the confluence of the posterior and lateral margins of the clypeus (L1) and the central point of the vertexal margin, delimited by a longitudinal line that divides the head capsule into two equal parts (L2; Fig. 1). Semilandmarks started at L1 and were sequentially sampled by length up to L2 (Fig. 1). We measured only half of the head due to bilateral symmetry. Landmarks and semilandmarks were digitized twice in all majors and minors for a total of 692 processed images to improve precision. Species in which minors had a posteriorly projected nuchal collar prevented us from directly visualizing L2. In these cases, we chose to omit this head region given that it did not correspond to the vertexal margin, and the L2 was placed anterior to the neck constriction.

All geometric morphometric analyses were performed in R 4.0.2 (R Core Team, 2020) using the GEOMORPH package v. 3.3.1 (Adams et al., 2020). Landmark and semilandmark coordinates were aligned using generalized Procrustes analysis (GPA), in which the specimens were translated to a common location, scaled to unit centroid size, and optimally rotated using the least-squares criterion (Rohlf & Slice, 1990). GPA was performed with two different approaches: (1) grouping major and minor workers to visualize the distribution of both subcastes in the morphospace and (2) separating major and minor workers as two distinct datasets to describe the morphospace occupied by each subcastes considering differences among species.

To assess errors during data acquisition, we performed a regression for Procrustes shape variables (Adams & Collyer, 2018; Goodall, 1991) with a residual randomization permutation procedure (Collyer et al., 2015; Collyer & Adams, 2018, 2020; see also Anderson & ter Braak, 2003 for further details) for the repeated measures between the original data set and a replicate to test whether the variation was significant among species (expected result) or within species (which would indicate errors in the acquisition of semi- and landmarks). This process was performed in two steps, first for the major workers' dataset and then for the minor workers' dataset.

To test the influence of allometry (within and between majors and minors), we used regression for Procrustes shape variables of independent contrasts of shape (Procrustes coordinates) on independent contrasts of size (centroid size), and statistical significance was assessed using residual randomization permutation procedure. We used the residual scores to remove the allometric effects to verify the main patterns of morphological variation between majors and minors. All subsequent analyses were performed without correcting for allometry. Differences considering majors' and minors' shape and distribution were tested using a regression for Procrustes shape variables with residual randomization permutation procedure.

Principal component analyses (PCAs) were performed to visualize and describe the distribution of subcastes and species shapes along different axes in tangent space. Thin-plate spline deformation grids (Bookstein, 1991) were employed to describe the shape differences visually. We chose not to perform a canonical variate analysis since the PCA was sufficient to indicate patterns of difference between-group variance compared to within-group variance so that there would be no need to apply procedures to maximize those differences.

Phylogenetic analysis

Although the complete tree by Economo et al. (2015) had 267 *Pheidole* species, they only estimated divergence times for a subset of 65 species because of computational constraints. Given that
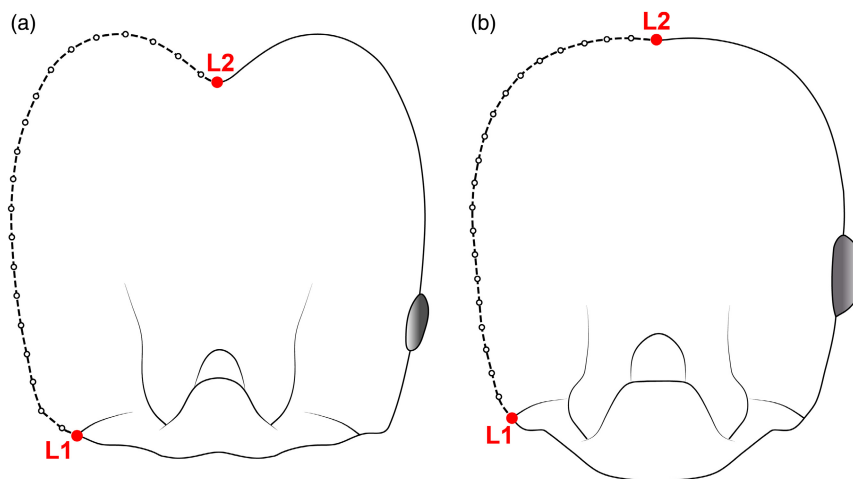

Figure 1 Position of landmarks (red dots) and semilandmarks (open dots) on the head of (a) major and (b) minor workers of *Pheidole*.

some of our analyses required a timetree, we sought to maximize the phylogenetic breadth of our analyses by fixing the 267-species topology obtained by Economo et al. (2015). The corresponding divergence times were only estimated using the RelTime method (Tamura et al., 2012) as implemented in MEGA7 (Kumar et al., 2016). We used a GTR+Γ5 model of evolution and eliminated all positions with less than 95% site coverage.

Phenograms were generated to assess head shape evolution, considering its geographic patterns (West Hemisphere and East Hemisphere), in a phylogenetic context, first plotting the main PCs scores on the phylogenetic tree, independently for each PC and centroid size. The phenogram was generated in PHY-TOOLS package v0.6-44 (Revell, 2012).

We also performed disparity-through-time plots (DTTs), as implemented in GEIGER v. 2.0.6 (Harmon et al., 2008), to explore the occupation of morphospace during *Pheidole* diversification (Harmon et al., 2003). This analysis compares the observed route of morphological diversification within majors and minors to what is usually expected from a Brownian motion (BM) model in multivariate space (Harmon et al., 2003, 2008). Through DTT, we can verify how a given clade, during its diversification, partitioned the occupied morphological space (Harmon et al., 2003). The difference between the observed and expected trajectories under the null BM model is quantified by the morphological disparity index (MDI), which is the sum of the deviations resulting from the trajectory observed in the DTT and its relation to those generated by the null model (Harmon et al., 2003). The index is then compared with the distribution of values obtained through the BM model simulations. Subsequently, it is tested to obtain significant deviations from the null model (Slater et al., 2010). Two possible results are obtained, one in which MDI > 0, indicating that the morphological variation within-clade is generally greater than expected in the null model, or MDI < 0, which implies that morphological variation is more dominant among-clades than expected in the null model, thus suggesting possible adaptive radiation (Murrell, 2018).

Rate shifts in morphological trait evolution among lineages were assessed using the "models of trait macroevolution on trees" approach (MOTMOT) implemented in MOTMOT package v. 1.1.2 (Thomas & Freckleton, 2012). This method is based on independent phylogenetic contrasts and allows for assessing patterns in the tempo of continuous trait evolution, showing the number and positions of rate changes in the phylogeny (Thomas & Freckleton, 2012). We used the first two PCs scores (see Duran & Pie, 2015; Hedrick et al., 2020; Pie et al., 2017; Sansalone et al., 2018) and the Csize (i.e., the centroid size as a proxy to describe variation in body size) to test shifts in morphological evolution. We performed these tests using the *transformPhylo.ML* function for PC1, PC2, and Csize individually, employing the trait medusa algorithm 1 (tm1), which does not specify the location of any shifts *a priori* (see Thomas & Freckleton, 2012 for further details). The maximum number of rate shifts was set to five.

Results

Measurement error during landmark acquisition was minimal, given that variation among individuals was substantially higher than among replicates for majors (SS = 0.393, $P = 0.652$) and minors (SS = 0.629, $P = 0.279$). On the other hand, we found a significant effect of size on shape between majors and minors, and within majors and minors (Table S2). After accounting for allometry, all PCAs successfully summarized shape variation in the studied datasets, with the first two PCs explaining more than 70% of the observed variation (Fig. 2a–d).

For majors, the first two PCs explained 50.11% and 27.13% of the variance in the dataset, respectively (Fig. 2c). Positive PC1 scores indicate longer heads, with the lateral margin diverging toward the vertex, angled vertexal lobes, and deeper vertexal depressions. In contrast, negative scores correspond to shorter heads, convex lateral margins, and shallow vertexal depressions (Fig. 2c). PC2 positive scores are related to rounded lateral margins and shallow vertexal depressions, whereas negative scores indicate a relatively flat margin and deep vertexal depression (Fig. 2c). Interestingly, WH *Pheidole* majors seem more diverse in their shape on PC1 than EH *Pheidole* (Fig. 2c), while an opposite trend was observed for PC2 (Fig. 2c). These patterns of shape differences between WH and EH species were corroborated in the Procrustes analysis of variance (ANOVA), which demonstrated significant differences between WH and EH majors (Table S3).

The first two PCs accounted for 78.86% and 10.90% of the observed variance for minors, respectively (Fig. 2d). Positive scores of PC1 are related to shorter heads, with the lateral margin diverging toward the vertex. In contrast, negative scores described longer heads with smoothly rounded sides, which was more common in species with a nuchal collar (Fig. 2d). Positive PC2 scores were associated with more rounded heads, whereas negative scores are related to divergent and flatter sides of the head, both common to shorter heads (Fig. 2d). There were also shape differences between EH and WH *Pheidole* minors, with the former showing more positive PC2 scores on average.

The PCA simultaneously including majors and minors allowed assessing interspecific variation within and among subcastes, showing strong allometry. When the allometric impact was accounted for, there was a clear separation between workers' subcastes on PC1 (80.13%), wherein majors and minors tended to have positive or negative scores, respectively (Fig. 2a). A PCA without accounting for allometry was markedly different (Fig. 2b), with a large superposition of majors and minors in the morphospace. Negative and positive scores had a similar morphological interpretation, as before, for PC1 and PC2, which accounted for 71.89% and 14.78% of the variance, respectively. A Procrustes ANOVA showed significant differences between their shapes despite the overlap among majors and minors without accounting for allometry (Table S2).

In a phylogenetic context, size and shape variation in PC1 and PC2 for worker subcastes revealed morphological divergence for closely related species (Fig. 3). The phenogram (Fig. 3) showed phylogenetic trends, suggesting an evolutionary convergence of head shape in most lineages, in both subcastes, and between the WH and EH specimens. The same patterns are demonstrated when analyzing the centroid size (Fig. 3). For minors, PC2 and Csize show a more distinct division into two
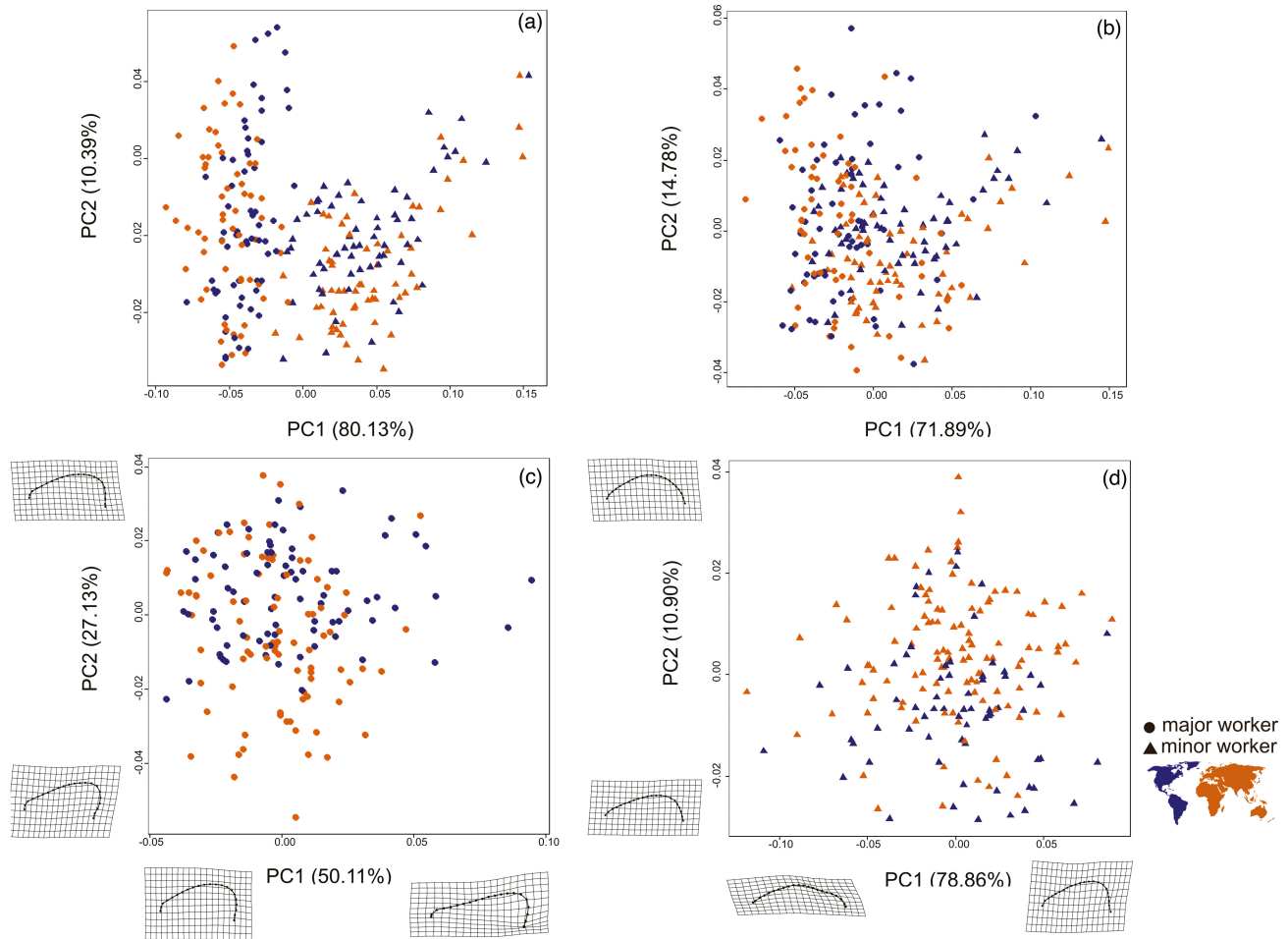
Figure 2 Morphospace of majors and minor workers combined with allometry (a) and without its effect (b); as well as majors (c) and minors (d) isolated. The PC1 and PC2 axes include the thin-plate spline, which illustrates the contour of the left side of the *Pheidole* worker heads generated by the grid lines distortion towards the maximum negative and positive value in the respective PC axes.

morphological groups, with little overlap between them (Fig. 3), while majors do not present this same pattern.

Once the relative position of each species and subcastes are understood within their morphospace, we explored how their rate of morphological evolution varied among lineages and over time. A consistent pattern was uncovered when variation in rates through time was assessed based on DTT plots. Our results indicated higher levels of disparity than expected by chance (MDI > 0) in Csize, PC1, and PC2 for the majors and PC2 for the minors (Fig. 4). In general, majors presented faster rates of morphological evolution near the present, especially on PC1. Nevertheless, these results suggest heterogeneity through time in rates of morphological changes evolution with more disparity near the present (Fig. 4). Minors showed higher differences in PC2 when compared with majors.

MOTMOT analyses detected frequent variations in rates of morphological changes evolution, both over time and among lineages (Fig. 5). For Csize, PC1, and PC2, MOTMOT identified nine rate shifts related to majors and seven to minors

(Fig. 5). Most of these shifts are associated with six acceleration events in majors and minors. All the identified decelerations in rates of morphological changes were observed in clades with more than three species, whereas the accelerations were more frequent in recent individual branches. For majors, shifts were more frequently associated with the WH (six accelerations and three decelerations; Fig. 5). For minors, these shifts were more frequent in the EH (four accelerations; Fig. 5), with a single related positive shift in *Pheidole californica* Mayr (Fig. 5, shifts 4 and 12). These results suggested an unusual and unequal condition for the evolution of morphological changes in these regions. Additionally, shift 6 in PC2 of majors and shift 15 in PC2 of minors are associated with Australasia and New Guinea species and a few Malagasy species. These shifts in Australasia and New Guinea clades correlate with high net diversification rates (see Economo et al., 2019 for further explorations of these points).

The age of rate shifts varied considerably yet showed some temporal congruence among the estimated ages for majors and
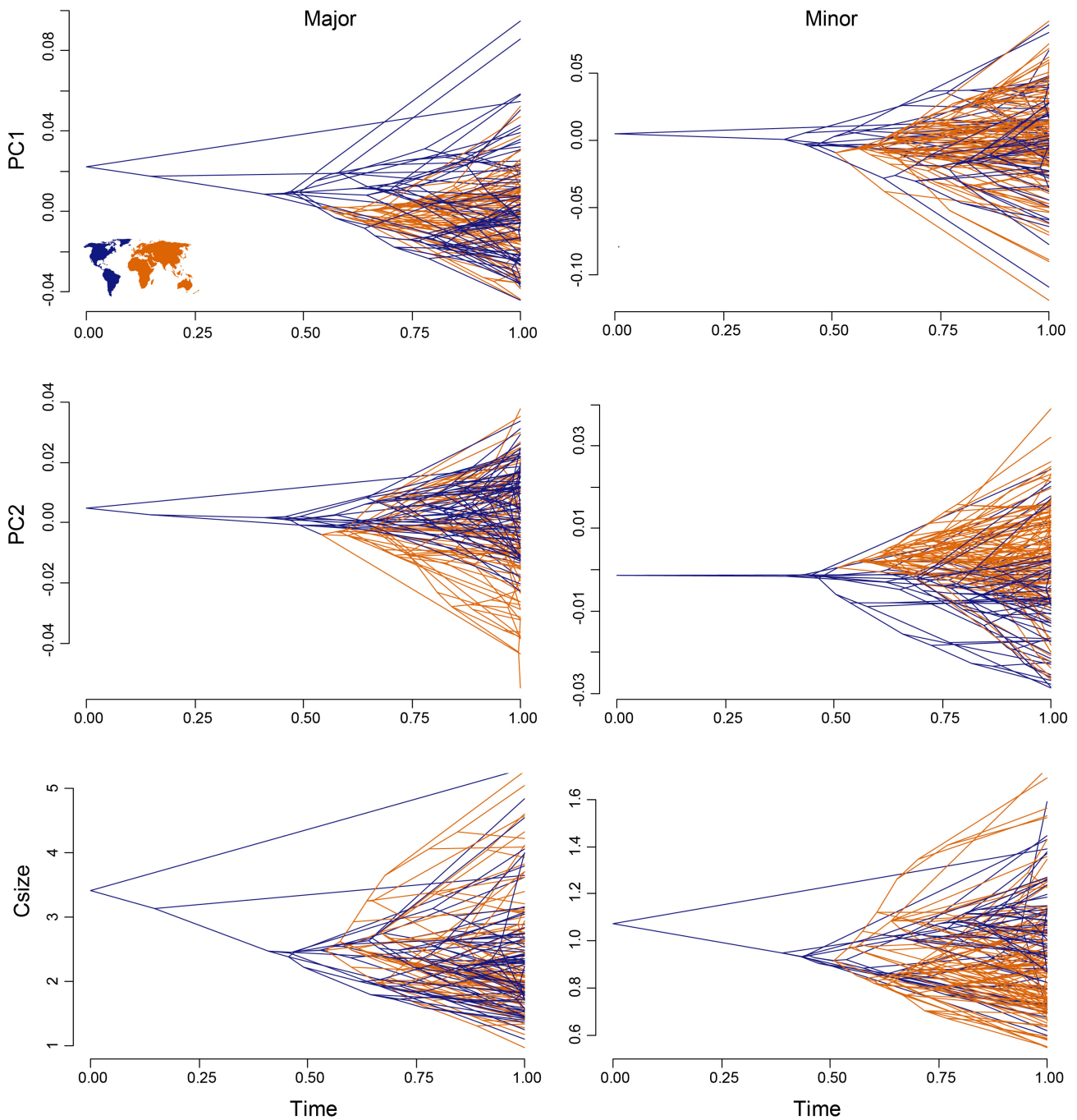
Figure 3 Phenogram of majors and minor workers of scores from the first two principal component axes and Csize (i.e., proxy of the head size). The relative time (*x*-axis) represents the approximate age of *Pheidole* clades to the present. Shape variation corresponds to the PCs scores and Csize (*y*-axis) and indicates divergences among linages evenly distributed through time (*x*-axis). Divergences are evident in branches that deviate from most branches sharing the same node in the phylogeny.

minors (Fig. 6). The highest concentration of shifts is related to the Miocene, with five clade shifts (1, 2, 11, 14, and 15) and two single shifts (4, 12) for Csize in majors and minors as well in PC2 of minors (Fig. 6). Only three shifts were

unrelated to the Miocene (PC1 in majors and minors, and PC2 in majors; Fig. 6). The most recent events were related to the Holocene in PC1 for each subcaste (shifts 5 and 13) and in PC2 for majors (shift 9; Fig. 6).
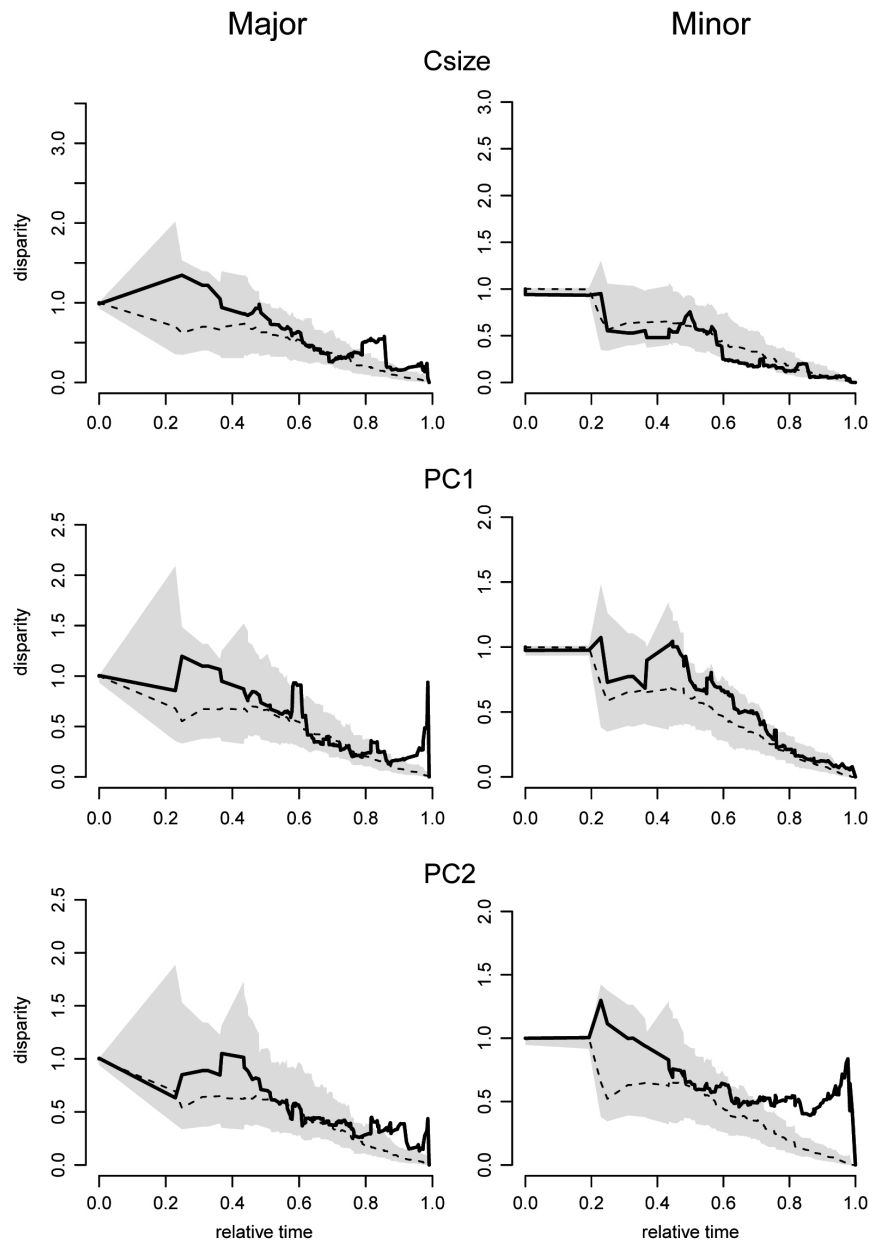
Major   Minor

Figure 4 Relative disparity-through-time (DTT) plots of PCs scores and Csize (i.e., a proxy of the *Pheidole* worker head size) in which the dashed line represents the expected under a null model of Brownian motion, the gray area corresponds to its 95% confidence interval, and the solid line indicated the observed disparity in the data.

Discussion

The evolutionary origin of exaggerated morphological structures provides a window into the versatility and limits of developmental programs (Emlen, 2001; Emlen & Nijhout, 2000; Shingleton & Frankino, 2013). Examples of exaggerated structures are found in many animal lineages (e.g., Gould, 1974) but seem to be particularly common in insects, such as stalk-eyed flies (Vasconcelos et al., 2019), lucanid beetles (Romiti et al., 2015), and several ant lineages (e.g., Blanchard

et al., 2020; Boudinot et al., 2021; Sarnat et al., 2017). Although exaggerated traits are most likely to be associated with males and be under sexual selection (e.g., weapons used for fights and/or display; see Emlen, 2008 for further discussion), several cases of such morphologies are present in females (e.g., Matsuura, 2006), as well as not being sexually selected (e.g., Bro-Jørgensen, 2007). Termites (Miura & Maekawa, 2020), ants (Oster & Wilson, 1978), social aphids (Stern & Foster, 1997), and thrips (Crespi et al., 1997) are some of the lineages in which individuals within the colony may
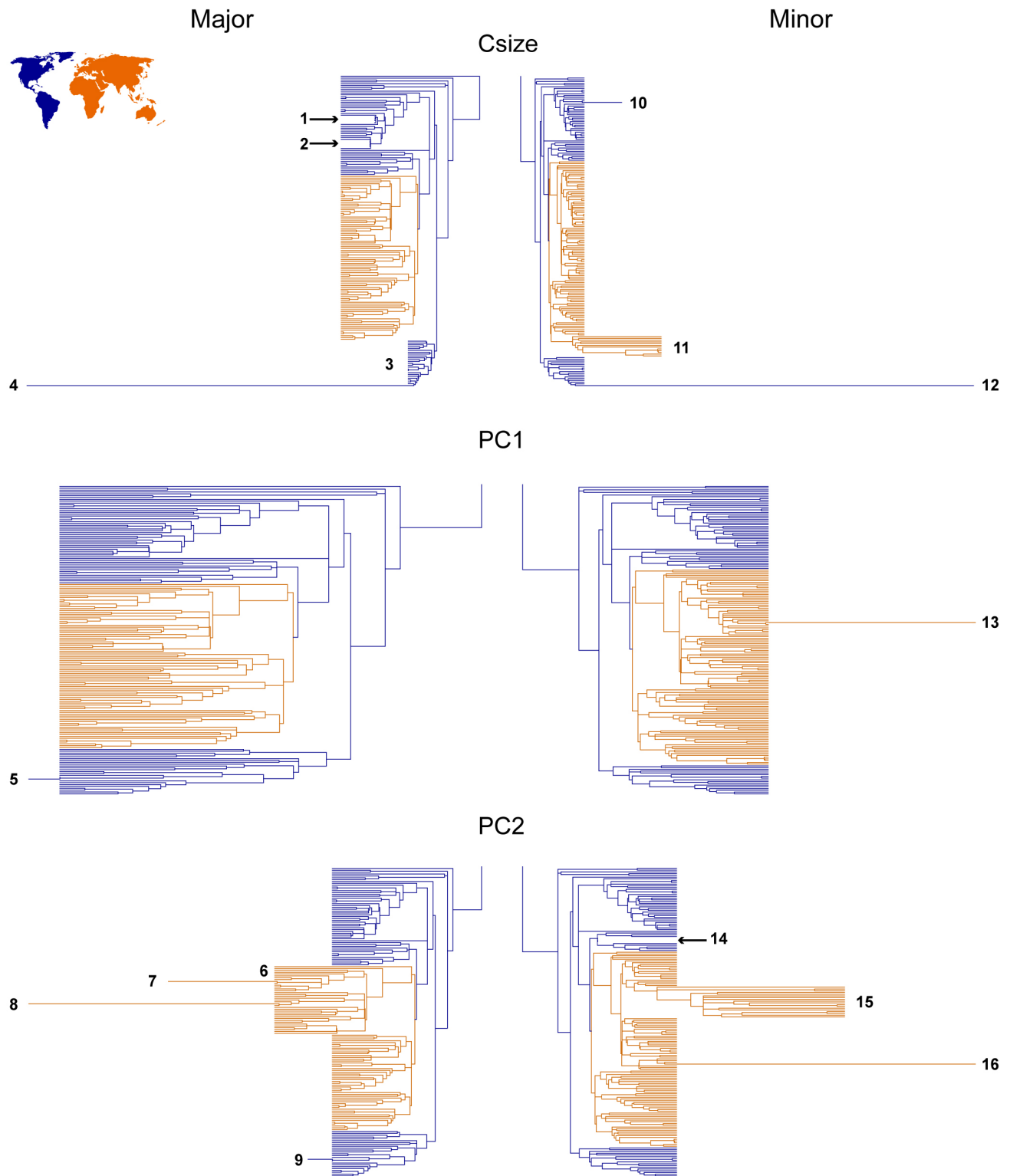
Figure 5 Shifts in rates of head evolution along with the first two PCs and the Csize (i.e., a proxy of the *Pheidole* worker head size) based on MOTMOT method (model = tm1). Numbers (1–16) indicate accelerations and decelerations in rates of morphological evolution demonstrated as changes in the branch lengths (x-axis) which are scaled according to the relative rates of evolution.
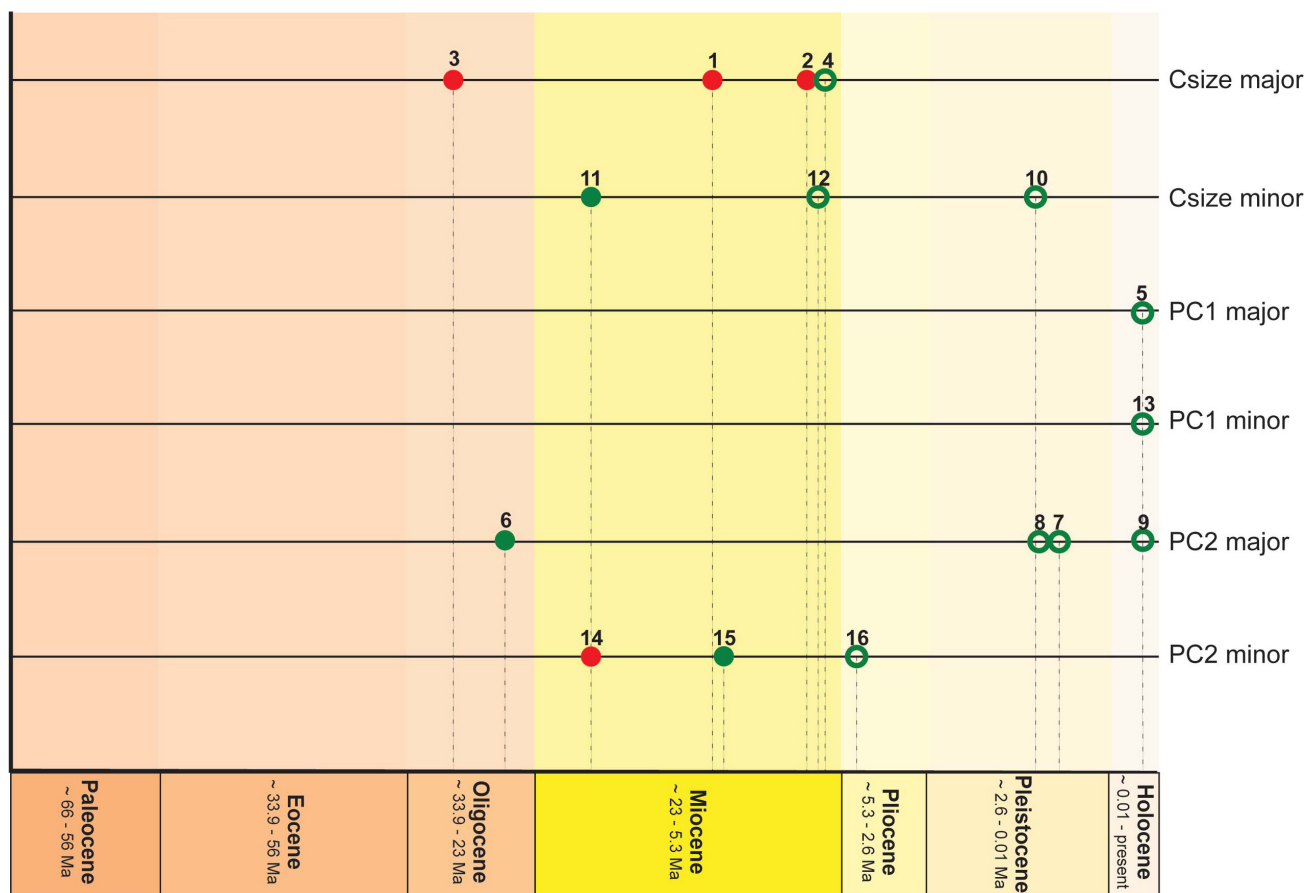
Figure 6 The geological periods (including the name of the geological era and its estimated duration period) for phylogenetic shifts are estimated by MOTMOT, and numbers (1–16) represent the same shifts shown in Fig. 5. The green dots indicate accelerations, and the red indicates decelerations. The open dots indicate the single species acceleration/deceleration, and the closes refer to clades acceleration/deceleration. The number corresponds to the MOTMOT phylogeny indicates. Csize was used as a proxy for *Pheidole* worker head size.

possess exaggerated morphological structures that were not driven by sexual selection. Instead, exaggerated morphologies in these groups are primarily associated with defense (e.g. mandibles, legs, and the presence of horn-like structures). However, the extent to which such non-sexually selected traits vary and evolve in social groups with conspicuous dimorphism remains poorly understood.

Here, we explored how head shape evolves in the context of a dimorphic worker caste, describing its main patterns, both over time and among lineages. We demonstrate distinct morphological patterns and evolutionary routes related to head shape in minor and major workers of *Pheidole*, with significant morphological changes for each subcaste for their geographical distribution (West and East Hemisphere). Also, our results showed that closely related species might diverge considerably in morphospace (Fig. 3). Moreover, West and East Hemisphere species converge in their occupation of phylomorphospace, presenting considerable overlap, with a few species exploring more peripheral regions (Fig. 3). We found disproportionately higher levels of disparity near the present, suggesting rate heterogeneity through time. Our results also indicated that most

morphological shifts occurred during the Miocene, with size shifts often preceding shape-related ones. However, clades with shifts in size are not congruent with those exhibiting shifts in the shape, suggesting that size and shape rate shifts are primarily uncoupled.

The allometric effect, which refers to the size-related changes in morphological traits, is crucial in caste differentiation (Trible & Kronauer, 2017; Wills et al., 2018). In this sense, allometry can play an important role in (1) differentiating distinct subcastes of workers during their development (i.e., ontogenetic allometry); (2) in morphological variations among individuals of the same colony, or between different colonies of the same species (i.e., static allometry); and (3) in morphological covariations within distinct lineages that share a common ancestor (i.e., evolutionary allometry; Klingenberg & Zimmermann, 1992). Our results indicate that evolutionary allometry is essential in evolving worker caste morphology, recognizing two morphologically distinct groups (Fig. 2a), representing majors and minors.

Additionally, contrary to what we predicted of non-size differences in the shape patterns of majors and minor workers,

our results showed that if the allometric effect is removed (Fig. 2b), there is considerable overlap between its occupied morphospace. Despite significant statistical differences between the two groups (Table S2), we note that the shapes attained by majors are also partially explored by minors. A similar result was obtained by Pie and Traniello (2007) using linear morphometrics. The authors argued that most of the variation in morphological divergence among *Pheidole* species could be attributed to allometric changes. The strong effect of size on *Pheidole* subcastes differentiation evidenced with linear and geometric morphometrics approaches is consistent with several publications which demonstrated that majors are determined late in larval development in a major–minor threshold (i.e., ontogenetic allometry), controlled by nutrition (more significant growth than minors; Rajakumar et al., 2012; Wheeler, 1911; Wheeler & Nijhout, 1983, 1984).

Few studies explore the evolution of size in *Pheidole* (e.g., Economo et al., 2015; Pie & Traniello, 2007; Pie & Tschá, 2013), even considering that this morphological attribute has been indicated as an essential factor in its evolution. Economo et al. (2015) explored the macroevolution and macroecology of *Pheidole* and showed that body size distribution has likewise converged across geographical regions. Economo et al. proposed that this morphological convergence is consistent with the global radiation of *Pheidole* structured by deterministic factors regulating diversification and diversity, with convergence occurring across independent radiations in each (East-West) hemisphere. Friedman et al. (2020) also found a morphological overlap of head and mesosoma size and shape considering *Pheidole* geographical radiations. Our results corroborate this pattern, showing an overlap in size over the East and West hemisphere fauna (Fig. 3).

Pie and Tschá (2013), also using linear morphometrics, found that the rate of size evolution is mainly constant over time. Additionally, the authors observed two broad negative shifts in body size evolution in majors and minors. Holley et al. (2016), using head measurements, found two positive and negative clade shifts. Our results indicated three negative clade shifts in the size of majors, and one positive in minors (Fig. 5). A discussion of the correspondence between these shifts is problematic considering that they may be affected by the unequal taxonomic breadth of the terminals between studies.

Considering shape variation, our results, in which PC1 and PC2 explain together ~80% of and ~90% of the variance for majors and minors, respectively, showed a similar pattern found for size. The occupation of the morphospace by majors and minor workers is consistent with our prediction that closely related lineages often diverged in shape and converged with distant lineages. Our results corroborate the findings of Friedman et al. (2020), with portions of the morphospace similarly occupied for geographically distinct *Pheidole* clades. Repeated and independent patterns of congruent evolution of similar morphological forms for different colonization processes are also present in other groups. A classic model of repeated evolution is the lizard genus *Anolis* (Mahler et al., 2013), in which each new colonization repeatedly generates the same ecomorphs, not closely related but converging for ecological, behavioral, or stochastic factors.

Nevertheless, the ant genus *Cephalotes*, a phylogenetically close lineage to *Pheidole*, also presents a similar pattern. *Cephalotes* is markedly recognized for their soldier morphology with four different ecomorphs, recognized for the head shape (Powell et al., 2020). Powell et al. (2020), exploring the evolution of head shape in *Cephalotes*, showed that each of these morphotypes evolved repeatedly. *Cephalotes* has experienced consistent gains and losses of each morphotype through its evolution, resulting in distant lineages converging for a common morphospace representing their ecomorphs (Powell et al., 2020). The selection of new optimal phenotype states that affect colony ergonomic efficiency in *Cephalotes* is strongly related to the head modifications in soldiers for colony defense. However, *Pheidole* has not evolved distinct ecomorphs as in *Cephalotes*. The behavioral and ecological implications of morphological divergences and convergences in *Pheidole* may primarily relate to how the muscles fit into the head and their volume. Thus, the head shape would provide strength for the mandibles, allowing new adaptations for defense and mechanical functions in the colony. However, more experiments need to be done relating form and function in *Pheidole* worker subcastes to understand how these morphological variations could affect the colony ergonomics.

We detected four statistically significant rate shifts (Fig. 5) in the morphological evolution of *Pheidole*. Additionally, DTT indicated higher levels of disparity, which suggested that morphological variation within-clade is generally greater than expected, contrary to what is predicted in a scenario of adaptive radiation (Murrell, 2018). Thus, head shape evolved relatively constant over time, and niche filling dynamics may not represent a limitation in the morphospace that dimorphic workers could explore as we predicted. This result is consistent with Pie and Tschá (2013), which showed that negative shifts are relatively common in ant evolution. Our results partially support our prediction that acceleration in the rate of shape diversification is associated with *Pheidole* adaptive radiation, in which we found two positive shifts (6 and 15, Fig. 6) related to the Australasia and New Guinea clades, coinciding with high net diversification rates (Economo et al., 2019).

Additionally, we found that shifts grouping in the Miocene are most frequently involved with decelerations (three shifts) than accelerations (two shifts) and coincides with the period between ~16 and 10 Myr. From the latter part of the Oligocene (26–27 Myr) to the middle Miocene (~15 Myr), there was a warming trend, which led to a reduction in the extent of the Antarctic ice (Miller et al., 1991; Wright et al., 1992). This phase of the warmest period, except for several brief glaciation periods (Wright & Miller, 1993), reached its ideal peak in the late middle Miocene (17–15 Myr) and was followed by a gradual cooling and re-establishment of a large ice-sheet in Antarctica (~10 Myr; Flower & Kennett, 1995; Vincent et al., 1985; Zachos et al., 2001). Waves of warmer, drier climate during this period, as well as the aridification over the Miocene and Pliocene boundary (8–6 Myr), were responsible for the emergence and establishment of the modern aspects and ranges of the grasslands (Axelrod, 1985; Cerling et al., 1997; Krasilov, 2003). Due to the wide association of granivorous species with open areas, the expansion of these environments may have

influenced its morphological diversification to occupy new explorable niches. Economo et al. ([2019](#)) suggested the importance of exploring the possible effects during this Miocene period on ant diversification since these climatic changes could be relevant to the increase of the evolutionary transitions of those lineages that occupied the tropics to the temperate zones.

Despite the previous studies on the diversification, integration, and quantification of *Pheidole* morphological attributes (e.g., Friedman et al., [2019](#), [2020](#); Pie & Traniello, [2007](#); Pie & Tschá, [2013](#); Tschá & Pie, [2019](#)), their implication in the massive diversity and prevalence of *Pheidole* over other lineages is far from being resolved. The advances to understand these diversification processes will be achieved by developing more powerful quantitative methods that use more diverse and better-quality data. The following steps would be to combine more significant representativeness of behavioral and ecological data and assess biomechanical data to understand the responses of these morphological patterns to different environmental demands in worker subcastes.

Acknowledgments

References

Adams, D. C., & Collyer, M. L. (2018). Multivariate phylogenetic comparative methods: Evaluations, comparisons, and recommendations. *Systematic Biology*, **67**(1), 14–31.

Adams, D. C., Collyer, M. L., & Kaliontzopoulou, A. (2020). *Geomorph: Software for geometric morphometric analyses. R package version 3.2.1.* https://cran.r-project.org/package=geomorph

Anderson, M. J., & ter Braak, C. J. F. (2003). Permutation tests for multi- factorial analysis of variance. *Journal of Statistical Computation and Simulation*, **73**, 85–113.

Antweb. (2019). Version 8.75.3. California Academy of Science. https://www.antweb.org. Accessed 2019.

Arrow, G. J. (1951). *Horned Beetles*. Dr. W Junk.

Axelrod, D. I. (1985). Rise of the grassland biome, central North America. *The Botanical Review*, **51**(2), 163–201.

Blanchard, B. D., Nakamura, A., Cao, M., Chen, S. T., & Moreau, C. S. (2020). Spine and dine: A key defensive trait promotes ecological success in spiny ants. *Ecology and Evolution*, **10**(12), 5852–5863.

Bolton, B. (2021). *An online catalog of the ants of the world*. https://antcat.org. Accessed 2021.

Bookstein, F. (1991). *Morphometric tools for landmark data: Geometry and biology.* Cambridge University Press.

Boudinot, B. E., Moosdorf, O. T. D., Beutel, R. G., & Richter, A. (2021). Anatomy and evolution of the head of *Dorylus helvolus* (Formicidae: Dorylinae): Patterns of sex-and caste-limited traits in the sausagefly and the driver ant. *Journal of Morphology*, **282**(11), 1616–1658.

Bro-Jørgensen, J. (2007). The intensity of sexual selection predicts weapon size in male bovids. *Evolution*, **61**, 1316–1326.

Cerling, T. E., Harris, J. M., MacFadden, B. J., Leakey, M. G., Quade, J., Eisenmann, V., & Ehleringer, J. R. (1997). Global vegetation change through the Miocene/Pliocene boundary. *Nature*, **389**(6647), 153–158.

Collyer, M. L., & Adams, D. C. (2018). RRPP: An R package for fitting linear models to high-dimensional data using residual randomization. *Methods in Ecology and Evolution*, **9**(7), 1772–1779. https://doi.org/10.1111/2041-210X.13029

Collyer, M. L., & Adams, D. C. (2020). *RRPP: linear model evaluation with Randomized Residuals in a Permutation Procedure, R package version 0.5.2.* https://cran.r-project.org/web/packages/RRPP/index.html

Collyer, M. L., Sekora, D. J., & Adams, D. C. (2015). A method for analysis of phenotypic change for phenotypes described by high-dimensional data. *Heredity*, **115**(4), 357–365.

Crespi, B. J., Carmean, and, D. A., & Chapman, T. W. (1997). Ecology and evolution of galling thrips and their allies. *Annual Review of Entomology*, **42**(1), 51–71.

Duran, A., & Pie, M. R. (2015). Tempo and mode of climatic niche evolution in Primates. *Evolution*, **69**(9), 2496–2506.

Economo, E. P., Huang, J. P., Fischer, G., Sarnat, E. M., Narula, N., Janda, M., Guénard, B., Longino, J. T., & Knowles, L. L. (2019). Evolution of the latitudinal diversity gradient in the hyperdiverse ant genus *Pheidole*. *Global Ecology and Biogeography*, **28**(4), 456–470.

Economo, E. P., Klimov, P., Sarnat, E. M., Guénard, B., Weiser, M. D., Beatrice Lecroq, L., & Knowles, L. (2015). Global phylogenetic structure of the hyperdiverse ant genus *Pheidole* reveals the repeated evolution of macroecological patterns. *Proceedings of the Royal Society B: Biological Sciences*, **282**(1798), 20141416.

Emlen, D. J. (2001). Costs and the diversification of exaggerated animal structures. *Science*, **291**(5508), 1534–1536.

Emlen, D. J. (2008). The evolution of animal weapons. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 387–413.

Emlen, D. J., & Nijhout, H. F. (2000). The development and evolution of exaggerated morphologies in insects. *Annual Review of Entomology*, **45**(1), 661–708.

Flower, B. P., & Kennett, J. P. (1995). Middle Miocene deepwater paleoceanography in the southwest Pacific: Relations with East Antarctic Ice-Sheet development. *Paleoceanography*, **10**(6), 1095–1112.

Friedman, N. R., Lecroq Bennet, B., Fischer, G., Sarnat, E. M., Huang, J. P., Knowles, L. L. K., & Economo, E. P. (2020). Macroevolutionary integration of phenotypes within and across ant worker castes. *Ecology and evolution*, **10**(17), 9371–9383.

Friedman, N. R., Remeš, V., & Economo, E. P. (2019). A morphological integration perspective on the evolution of dimorphism among sexes and social insect castes. *Integrative and Comparative Biology*, **59**(2), 410–419.

Goodall, C. (1991). Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society: Series B (Methodological)*, **53**(2), 285–321.

Gould, S. J. (1974). The origin and function of 'bizarre' structures: antler size and skull size in the 'Irish Elk', *Megaloceros giganteus*. *Evolution*, **28**, 191–220.

Harmon, L. J., Losos, J. B., Jonathan Davies, T., Gillespie, R. G., Gittleman, J. L., Bryan Jennings, W., Kozak, K. H., McPeek, M. A., Moreno-Roark, F., Near, T. J., Purvis, A., Ricklefs, R. E., Schluter, D., Schulte II, J. A., Seehausen, O., Sidlauskas, B. L., Torres-Carvajal, O., Weir, J. T., & Mooers, A. Ø. (2010). Early bursts of body size and shape evolution are rare in comparative data. *Evolution*, **64**(8), 2385–2396.

Harmon, L. J., Schulte, J. A., Larson, A., & Losos, J. B. (2003). Tempo and mode of evolutionary radiation in iguanian lizards. *Science*, **301**(5635), 961–964.

Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E., & Challenger, W. (2008). GEIGER: Investigating evolutionary radiations. *Bioinformatics*, **24**(1), 129–131.

Hedrick, B. P., Dickson, B. V., Dumont, E. R., & Pierce, S. E. (2020). The evolutionary diversity of locomotor innovation in rodents is not linked to proximal limb morphology. *Scientific Reports*, **10**(1), 1.

Hölldobler, B., & Wilson, E. O. (2009). *The superorganism: the beauty, elegance, and strangeness of insect societies*. WW Norton & Company.

Holley, J. A. C., Moreau, C. S., Laird, J. G., & Suarez, A. V. (2016). Sub-caste-specific evolution of head size in the ant genus *Pheidole*. *Biological Journal of the Linnean Society*, **118**(3), 472–485.

Klingenberg, C. P., & Zimmermann, M. (1992). Static, ontogenetic, and evolutionary allometry: a multivariate comparison in nine species of water striders. *The American Naturalist*, **140**(4), 601–620.

Krasilov, V. A. (2003). *Terrestrial palaeoecology and global change (No. 1)*. Pensoft Pub.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**, 1870–1874.

Liu, C., Sarnat, E. M., Friedman, N. R., Hita Garcia, F., Darwell, C., Booher, D., Kubota, Y., Mikheyev, A. S., & Economo, E. P. (2020). Colonize, radiate, decline: Unraveling the dynamics of island community assembly with Fijian trap-jaw ants. *Evolution*, **74**(6), 1082–1097.

Mahler, D. L., Ingram, T., Revell, L. J., & Losos, J. B. (2013). Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science*, **341**(6143), 292–295.

Matsuura, K. (2006). A novel hypothesis for the origin of the sexual division of labor in termites: Which sex should be soldiers? *Evolutionary Ecology*, **20**(6), 565–574.

Mertl, A. L., & Traniello, J. F. (2009). Behavioral evolution in the major worker sub-caste of twig nesting *Pheidole* (Hymenoptera: Formicidae): Does morphological specialization influence task plasticity? *Behavioral Ecology and Sociobiology*, **63**(10), 1411–1426.

Miller, K. G., Wright, J. D., & Fairbanks, R. G. (1991). Unlocking the ice house: Oligocene- Miocene oxygen isotopes, eustasy, and margin erosion. *Journal of Geophysical Research: Solid Earth*, **96**(B4), 6829–6848.

Miura, T., & Maekawa, K. (2020). The making of the defensive caste: Physiology, development, and evolution of the soldier differentiation in termites. *Evolution & Development*, **22**(6), 425–437.

Molet, M., Wheeler, D. E., & Peeters, C. (2012). Evolution of novel mosaic castes in ants: Modularity, phenotypic plasticity, and colonial buffering. *The American Naturalist*, **180**(3), 328–341.

Moreau, C. S. (2008). Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution*, **48**(1), 224–239.

Murrell, D. J. (2018). A global envelope test to detect non-random bursts of trait evolution. *Methods in Ecology and Evolution*, **9**(7), 1739–1748.

Oster, G. F., & Wilson, E. O. (1978). *Caste and ecology in the social insects*. Princeton University Press.

Parsons, K. J., & Robinson, B. W. (2006). Replicated evolution of integrated plastic responses during early adaptive divergence. *Evolution*, **60**(4), 801–813.

Pie, M. R., Campos, L. L., Meyer, A. L., & Duran, A. (2017). The evolution of climatic niches in squamate reptiles. *Proceedings of the Royal Society B: Biological Sciences*, **284** (1858), 20170268.

Pie, M. R., & Traniello, J. F. A. (2007). Morphological evolution in a hyperdiverse clade: the ant genus *Pheidole*. *Journal of Zoology*, **271**(1), 99–109.

Pie, M. R., & Tschá, M. K. (2013). Size and shape in the evolution of ant worker morphology. *PeerJ*, **1**, e205.

Powell, S. (2016). A comparative perspective on the ecology of morphological diversification in complex societies: Nesting ecology and soldier evolution in the turtle ants. *Behavioral Ecology and Sociobiology*, **70**(7), 1075–1085.

Powell, S., Price, S. L., & Kronauer, D. J. (2020). Trait evolution is reversible, repeatable, and decoupled in the soldier caste of turtle ants. *Proceedings of the National Academy of Sciences of the United States of America*, **117** (12), 6608–6615.

R Core Team (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. http://www.R-project.org/

Rajakumar, R., San Mauro, D., Dijkstra, M. B., Huang, M. H., Wheeler, D. E., Hiou-Tim, F., Khila, A., Cournoyea, M., & Abouheif, E. (2012). Ancestral developmental potential facilitates parallel evolution in ants. *Science*, **335**(6064), 79–82.

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**(2), 217–223.

Rohlf, F. J. (2021). *tpsDig2. 31. Department of Ecology and Evolution at State University of New York*. Stony Brook.

Rohlf, F. J., & Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology*, **39**(1), 40–59.

Romiti, F., Tini, M., Redolfi De Zan, L., Chiari, S., Zauli, A., & Carpaneto, G. M. (2015). Exaggerated allometric structures in relation to demographic and ecological parameters in *Lucanus cervus* (Coleoptera: Lucanidae). *Journal of Morphology*, **276** (10), 1193–1204.

Sansalone, G., Colangelo, P., Kotsakis, T., Loy, A., Castiglia, R., Bannikova, A. A., Zemlemerova, E. D., & Piras, P. (2018). Influence of evolutionary allometry on rates of morphological evolution and disparity in strictly subterranean moles (Talpinae, Talpidae, Lipotyphla, Mammalia). *Journal of Mammalian Evolution*, **25**(1), 1–4.

Sarnat, E. M., Friedman, N. R., Fischer, G., Lecroq-Bennet, B., & Economo, E. P. (2017). Rise of the spiny ants: diversification, ecology and function of extreme traits in the hyperdiverse genus *Pheidole* (Hymenoptera: Formicidae). *Biological Journal of the Linnean Society*, **122**(3), 514–538.

Shingleton, A. W., & Frankino, W. A. (2013). New perspectives on the evolution of exaggerated traits. *BioEssays*, **35**(2), 100–107.

Slater, G. J., Price, S. A., Santini, F., & Alfaro, M. E. (2010). Diversity versus disparity and the radiation of modern cetaceans. *Proceedings of the Royal Society B: Biological Sciences*, **277**(1697), 3097–3104.

Stern, D. L., & Foster, W. A. (1997). The evolution of sociality in aphids: A clone's-eye view. In J. C. Coohoe, & B. J. Crespi (Eds.), *The evolution of social behavior in insects and arachnids* (pp. 150–165). Cambridge University Press.

Tamura, K., Battistuzzi, F. U., Billing-Ross, P., Murillo, O., Filipski, A., & Kumar, S. (2012). Estimating divergence times in large molecular phylogenies. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 19333–19338.

Thomas, G. H., & Freckleton, R. P. (2012). MOTMOT: Models of trait macroevolution on trees. *Methods in Ecology and Evolution*, **3**(1), 145–151.

Traniello, J. F. A. (2010). *Pheidole*: sociobiology of a highly diverse genus. In M. D. Breed, & J. Moore (Eds.), *Encyclopedia of animal behavior* (pp. 699–706). Academic Press.

Trible, W., & Kronauer, D. J. (2017). Caste development and evolution in ants: It's all about size. *Journal of Experimental Biology*, **220**(1):53–62.

Tschá, M. K., & Pie, M. R. (2019). Correlates of ecological dominance within *Pheidole* ants (Hymenoptera: Formicidae). *Ecological Entomology*, **44**(2), 163–171.

Vasconcelos, A. C. O., Carvalho, C. J. B., & Pie, M. R. (2019). Static allometry in two species of neotropical stalk-eyed fly. *Journal of Zoology*, **309**(1), 43–49.

Vincent, E., Killingley, J. S., & Berger, W. H. (1985) Miocene oxygen and carbon isotope stratigraphy of the tropical Indian Ocean. In: J. P. Kennett (ed.), *The Miocene Ocean: Paleoceanography and biogeography* Vol. **163** (pp. 103–130). Geological Society of America Memoir.

Wheeler, W. M. (1911). The ant-colony as an organism. *Journal of Morphology*, **22**(2), 307–325.

Wheeler, D. E., & Nijhout, H. F. (1983). Soldier determination in *Pheidole bicarinata*: effect of methoprene on caste and size within castes. *Journal of Insect Physiology*, **29**(11), 847–854.

Wheeler, D. E., & Nijhout, H. F. (1984). Soldier determination in *Pheidole bicarinata*: inhibition by adult soldiers. *Journal of Insect Physiology*, **30**(2), 127–135.

Wills, B. D., Powell, S., Rivera, M. D., & Suarez, A. V. (2018). Correlates and consequences of worker polymorphism in ants. *Annual Review of Entomology*, **63**, 575–598.

Wilson, E. O. (2003). *Pheidole in the New World: A dominant, hyperdiverse ant genus*, Vol. **1**. Harvard University Press.

Wright, J. D., & Miller, K. G. (1993). Southern ocean influences on late Eocene to Miocene deepwater circulation. In J. P. Kennet, & D. A. Warnke (Eds.), *The Antarctic Paleoenvironment: A perspective on global change* (pp. 1–25). American Geophysical Union.

Wright, J. D., Miller, K. G., & Fairbanks, R. G. (1992). Early and middle Miocene stable isotopes: Implications for deepwater circulation and climate. *Paleoceanography*, **7**(3), 357–389.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., & Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, **292**(5517), 686–693.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Allometry results for major, minors, and combined dataset. The values in bold are statistically significant.

Table S2. Results of Procrustes analysis of variance considering shape and distribution for majors and minor workers. For the combined data, distribution and subcastes were considered. The values in bold are statistically significant.

Table S3. Species and corresponding codes referring to the images available in AntWeb and their distribution in the Western Hemisphere (WH) or Eastern Hemisphere (EH).