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5 Article type : Original Research

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11 **Macroevolutionary integration of phenotypes within and across ant worker**
12 **castes**

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/ECE3.6623](https://doi.org/10.1002/ECE3.6623)

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30

31 **Abstract**

32

33 Phenotypic traits are often integrated into evolutionary modules: sets of organismal parts that
34 evolve together. In social insect colonies the concepts of integration and modularity apply to sets
35 of traits both within and among functionally and phenotypically differentiated castes. On
36 macroevolutionary timescales, patterns of integration and modularity within and across castes
37 can be clues to the selective and ecological factors shaping their evolution and diversification.
38 We develop a set of hypotheses describing contrasting patterns of worker integration and apply
39 this framework in a broad (246 species) comparative analysis of major and minor worker
40 evolution in the hyperdiverse ant genus *Pheidole*. Using geometric morphometrics in a
41 phylogenetic framework, we inferred fast and tightly integrated evolution of mesosoma shape
42 between major and minor workers, but slower and more independent evolution of head shape
43 between the two worker castes. Thus, *Pheidole* workers are evolving as a mixture of intra- and
44 inter-caste integration and rate heterogeneity. The decoupling of homologous traits across worker
45 castes may represent an important process facilitating the rise of social complexity.

46

47 **Keywords:**

48 Morphological integration, modularity, caste, dimorphism, *Pheidole*, ants, geometric
49 morphometrics

50

51 **Introduction**

52

53 The increase of morphological complexity following divergence in cellular function is a
54 repeating theme in the evolution of multicellular organisms (Wagner and Altenberg 1996). Given
55 cues regarding their developmental fate, cells and tissues express their identical genomes in
56 different ways to produce different traits and thus allow functional specialization. Morphological
57 integration can be considered the extent to which these traits vary in concert, either as a
58 continuation of their shared genetic or developmental origin, or as a unification of parts
59 contributing to a shared function and shaped by selection (Olson and Miller 1958; Klingenberg

60 2008). Sets of integrated traits covary as modules, between which covariation is weaker than
61 within (as in the primate cranium; Cheverud 1982).
62
63 Much as a single genome can underlie different cooperating tissues and traits within the same
64 organism, different traits are also produced among individuals using the same genome. Distinct
65 phenotypes are commonly observed in different sexes (Owens and Hartley 1998), or in
66 individuals adopting alternative reproductive tactics (Emlen et al. 2007) as a result of differential
67 selection. Eusocial insects reflect a major evolutionary transition whereby a unit of selection is
68 comprised of different individuals working together as part of an integrated colony-level
69 phenotype (Wheeler 1911; Hölldobler and Wilson 1990; Szathmáry and Smith 1995) and
70 understanding the evolution and function of these “superorganisms” is a major and enduring
71 interest of evolutionary biology (Oster and Wilson 1978; Seeley 1995; Holldobler and Wilson
72 2009). The castes of social insects can exhibit radically different traits from the same genome: a
73 female egg laid by the queen has the potential to develop into either another queen or a worker
74 caste individual. This phenotypic polymorphism allows functional specialization among
75 individuals in a colony and the rise of social complexity, the feature of eusociality that best
76 defines its potential for division of labor (Oster and Wilson 1978; Hölldobler and Wilson 1990).
77 While worker castes are an ancestral trait shared by nearly all extant ants, several lineages have
78 since evolved further division of labor among workers to form worker castes – known also as
79 subcastes (Wilson 1953; Hölldobler and Wilson 1990; Oster and Wilson 1978; Wills et al. 2017).
80 In the colonies of some species (e.g., *Solenopsis invicta*), worker castes exhibit polymorphism
81 mostly along a single allometric function – shape varies with size along a regular continuum
82 (Wilson 1953). However, for species in other genera (e.g., *Pheidole*, *Colobopsis*, *Carebara*,
83 *Cephalotes*, *Eciton*, *Acanthomyrmex*, *Pseudolasius*), variation reaches “complete dimorphism”
84 into distinct major worker and minor worker phenotypes (Wilson 1953). While there is some
85 contention over nomenclature within the myrmecological community (Urbani 2015), we refer to
86 minor workers and major workers (aka soldiers) as “worker castes” throughout following Wills
87 et al. (2017).
88
89 The evolution of complete dimorphism offers the potential for new dimensions of variation in
90 ants (Wilson 1953, Wills et al. 2017). If phenotypes are disintegrated among worker castes, this

91 can allow for greater functional specialization and different combinations of traits available to
92 the colony-level phenotype (Wilson 1953; Powell 2008; Powell 2009; Wills et al. 2017).
93 However, the evolution of specialized morphology in major workers may be biased by
94 developmental pathways that are shared with minors (Wheeler and Nijhout 1983; Wheeler and
95 Nijhout 1984, Wheeler 1991; Rajakumar et al. 2012), thus there could be limits to divergence
96 among homologous body parts across the different worker castes, or a shared pathway could lead
97 selection on one worker caste to result in a neutral change in the other.

98
99 The ecological and behavioral roles of polymorphic worker ants have long been a fascination of
100 social insect research (Wheeler 1911; Goetsch 1937; Wilson 1953, Oster and Wilson 1978;
101 Powell and Franks 2006; Powell 2008; Powell 2009; Powell 2016; Wills et al. 2017). Likewise,
102 the genomic and biochemical mechanisms underpinning caste differentiation is a central avenue
103 for understanding the evolution of social complexity (Wheeler 1991; Hughes et al. 2003,
104 Anderson et al. 2008; Molet et al. 2012; Rajakumar et al. 2012; Lillico-Ouachour and Abouheif
105 2017, Gospocic et al. 2017, Chandra et al. 2018). However, the macroevolutionary implications
106 of these processes—the patterns of integration and modularity that emerge across the
107 diversification of hundreds or thousands of lineages—are less well-studied in ants than other taxa,
108 although the topic is receiving increasing interest (Pie and Traniello 2007; Pie and Tschá 2013;
109 Holley et al. 2016; Powell 2016). These patterns, revealing the degree to which different traits
110 evolve independently within and among worker castes, may be an important clue to both the
111 selective forces driving evolution like ecological subspecialization among worker castes (Powell
112 and Franks 2006), and the potential constraints on evolution like restrictive developmental
113 limitations (as in Fritz et al. 2014). Furthermore, the differences in evolutionary rates among
114 different traits in the same worker castes, or the same trait in different worker castes, may reflect
115 aspects of the phenotype that are under strong selection because they underlie axes of ecological
116 divergence among species (Schluter 2000; Price et al. 2016).

117
118 Ants have colonized and evolved adaptations to many environments, and are among the most
119 abundant terrestrial organisms on the planet. Ants have also radiated to produce a diverse array
120 of morphologies in nearly every region they have colonized (Hölldobler and Wilson 1990). In
121 this study, we focus on overall body size, the relative body sizes of different parts, and the shapes

122 of the head and mesosoma. Body size has been shown to be a major axis of morphological
123 variation among ants (Pie and Traniello 2007, Price et al. 2016; Powell 2016). Previous studies
124 of functional morphology in ants have also focused on the head (e.g., Holley et al. 2016), which
125 contains the main apparatus for feeding (mouthparts, mandibles), manipulating objects
126 (mandibles), and sensation (eyes, antennae). If head shape is the primary focus of ecological
127 adaptation, this trait should evolve more rapidly than others during radiation. We also examine
128 the mesosoma, the main power center of the ant including muscles for bearing loads and moving
129 the legs. While the functional significance of external mesosoma shape is not well understood,
130 the shapes and relative sizes of different regions likely reflects investment in different muscle
131 groups that have functional implications. The sizes and positions of the sclerites (plates of the
132 cuticle which are partially captured here by our landmarks) are associated with homologous
133 attachment points underneath. For example, Keller et al. (2014) showed that the pronotal region
134 associated with the T1 sclerite houses the muscles that lift the head. Other regions of the
135 mesosoma contain stabilizing muscles, muscles to support the legs, and muscles to flex the
136 petiole (Lubbock 1881), all of which have obvious functional implications.

137 Relatively few studies have compared the tempo of evolution across different ant traits,
138 (but see Pie and Tschá 2013; Blanchard and Moreau 2017; Holley et al. 2016). If the shapes of
139 other traits such as the mesosoma (thorax) evolve more rapidly, this may be an indication that
140 they serve a greater functional role in ecological divergence than previously understood.
141 Likewise, if majors exhibit greater rates of change than minor workers, that may signal that their
142 functional role has changed often following the evolution of complete dimorphism, or that they
143 are important for achieving and maintaining ecological divergence among species.

144
145 To compare morphological integration and evolutionary rate of different worker castes and traits,
146 we focused on the ant genus *Pheidole*. The ants of this genus have, in the course of their
147 approximately 37 million year history, spread throughout 6 continents to produce more than
148 1000 described (and many more undescribed) species (Moreau 2008; Economo et al. 2015a).
149 Perhaps the most notable characteristic of species in this hyperdiverse genus is the clear
150 dimorphism of their workers: a major worker caste with enlarged heads is easily visible in all
151 species (indeed a third super-major form is also observed in some species) (Wilson 2003).
152 Behavioral studies have described different ecological roles for *Pheidole* worker castes, with

153 major workers performing more defense, food processing, and storage tasks than minor workers
154 (Wilson 1984; Tsuji 1990; Mertl and Traniello 2009; Huang 2010). The relatively consistent
155 body plan and caste structure of this genus make it an ideal clade for comparative studies of
156 morphology (Pie and Traniello 2007; Holley et al. 2016). The developmental basis of worker
157 caste differentiation in *Pheidole* has been well studied over the years (Wheeler and Nijhout 1983;
158 Wheeler and Nijhout 1984; Rajakumar et al. 2012; Lillico-Ouachour and Abouheif 2016;
159 Rajakumar et al. 2018), and recent work on the taxonomy, biogeography, and ecomorphology of
160 this group (Wilson 2003; Mertl and Traniello 2009, Muscedere and Traniello 2012; Sarnat and
161 Moreau 2011, Economo and Sarnat 2012, Economo et al. 2015b; Holley et al. 2016; Sarnat et al.
162 2017) make it an attractive model clade for evolutionary research on social insects.

163
164 Several previous studies on the macroevolution of *Pheidole* morphology are particularly relevant
165 for the current investigation. First, in an analysis before a *Pheidole* phylogeny was available, Pie
166 and Traniello (2006) analyzed morphology with linear measurements and found that size
167 differences explained most of the variation in *Pheidole* morphology across species, but majors
168 and minors showed divergent patterns of character correlation. Later, with the benefit of a
169 *Pheidole* phylogeny (Moreau 2008), Pie and Tschá (2013) showed that size varied more quickly
170 than shape variables based on linear morphometrics, but did not explicitly test for modularity and
171 integration. Holley et al. (2016) found that known ecological specialization of majors (seed
172 milling behavior in granivorous species) was related to divergence in head size between major
173 and minor worker castes (although enigmatically, due to a change in the minors), evidence that
174 independent evolution of the two worker castes in relation to ecology can occur. Finally, Sarnat
175 et al. (2017) tested hypotheses for the evolution of exaggerated thoracic spines, an unusual and
176 geographically restricted phenotype in *Pheidole*.

177
178 Despite the insights of these pioneering studies, a comprehensive picture of the roles of
179 integration, modularity, and rate heterogeneity in morphological evolution within and among
180 *Pheidole* castes has not emerged. Using landmark-based geometric morphometrics, and taking
181 advantage of recent progress on reconstructing the *Pheidole* phylogeny (Economo et al. 2015a;
182 Economo et al. 2019) which allows for a more taxonomically and geographically extensive
183 analysis, we perform the most morphologically and phylogenetically comprehensive analysis to-

184 date to attempt to infer a general picture of integration and modularity in size and shape in the
185 *Pheidole* worker castes.

186

187 To frame our study, we propose a set of hypotheses predicting different patterns of
188 morphological integration within and among castes in social insect colonies (see Figure 1). We
189 discuss this in terms of the head and mesosoma (thorax) of *Pheidole* worker castes, but it could
190 equally be applied to any morphological traits shared among castes, or indeed traits shared
191 among other differentiated phenotypes like sexes or reproductive strategies (Simpson et al. 2011).
192 First, different parts of the body *within* a worker caste may be more or less integrated. This
193 integration could reflect developmental biases or biomechanical constraints, for example a
194 specific change in head morphology may necessitate a specific change of the thoracic segments
195 that support or move the head. Second, *across* worker castes the same homologous body parts
196 could be more or less integrated. As different worker castes share not only genomes but
197 developmental pathways, it is plausible that selection on a trait in one worker caste could lead to
198 a change in another worker caste. For example, selection on elongation of the head of a minor
199 worker may lead to similar elongation in the major worker, even if there is no inclusive fitness
200 benefit to the change in the major worker. Or, each worker caste could vary independently
201 facilitating different functional roles in the colony.

202

203 We test these hypotheses by assessing the presence and pattern of integration of the head and
204 mesosoma within and among worker castes. First, we assess heterogeneity in rates of evolution
205 across body parts and worker castes; whether evolutionary change tends to follow a pattern in
206 which different parts or worker castes are hot or cold spots of change, or whether traits evolve at
207 similar rates within and among worker castes. Second, we look for patterns of modularity in
208 shape and size to test how well an evolutionary change in shape or size of one trait predicts the
209 shape and size of another trait within the same worker caste or in a different one. If there are
210 differences in evolutionary rates, we ask again whether those differences reflect characteristics
211 shared among homologous traits or among worker castes.

212

213 **Methods**

214

215 *Photographic Measurements*

216

217 All comparative studies reflect a compromise between depth of individual sampling within
218 species versus breadth across species. In this study we aimed to expand the latter to include as
219 many *Pheidole* species as possible. We acknowledge a drawback of this strategy, which is that
220 we cannot capture the size or shape range of individuals within each species. We measured a
221 total of 1164 specimens from 314 species, measuring an average of 2.18 major worker and 2.20
222 minor worker specimens per species; to maintain consistency between samples, all
223 measurements were performed by coauthor BL. Myrmecologists use high resolution montage
224 photographs to document ant diversity, following a standardized set of specimen positions that
225 display head and body features from a consistent angle as described by the online resource and
226 repository, AntWeb.org. We made a broad effort to photograph specimens from species used in
227 recent phylogenetic projects (Economato et al. 2015a), supplemented with photographs taken by
228 others and deposited on Antweb.org. We endeavored to collect data on both major and minor
229 workers whenever possible, however photographic data for both worker castes were only
230 available for 214 species or 68% of our total taxonomic sample. To account for potential focal
231 length issues when using 2D photographs taken with different optical systems, we landmarked
232 the same specimen 100 times under six different magnifications. A focal length warping effect
233 was observable but was non-significant, and was within the range of intraspecific variation.

234

235 For each specimen, we placed landmarks using the three standard photographic angles: head
236 view, dorsal view, and profile view. We collected landmarks from features that were consistently
237 in the plane of the camera angle. Specifically, we placed 11 landmarks on the dorsal view of the
238 head (Appendix 1) and 6 landmarks on the profile view of the body (Appendix 2; all located on
239 the mesosoma; hereafter head, mesosoma; see Figure 2). To capture information on the posterior
240 head shape, we also included a set of 6 sliding semi-landmarks (7 in major workers) from
241 landmark 3 to 11 (Figure 2). The landmarks on the left side of the head were reflected bilaterally
242 to produce the curve on the right side of the head between landmarks 11 and 1. Fixed landmarks
243 on opposite sides of the head were reflected and averaged to force object symmetry.

244 While these landmarks omit several features that vary among *Pheidole* taxa, and those typically
245 used in myrmecology research and taxonomy (Pie and Traniello 2007), this was unavoidable due

246 to the constraints of choosing homologous landmarks in positions that are not occluded by
247 nearby features (e.g., the anterior pronotum is often occluded by the posterior head lobes).

248

249 *Geometric Morphometrics*

250

251 We performed a generalized Procrustes alignment on each set of landmarks using the R package
252 *geomorph*, employing separate analyses for major and minor workers (Adams and Otárola-
253 Castillo 2013; version 3.0.7). Specimens showing greater than expected distance from the
254 Procrustes mean (i.e., above the upper quartile) were inspected for improper scale entry or
255 landmark order/placement. Photos for which improper specimen positioning was observed were
256 removed from the data set (< 1% of specimens studied). Within each species, we calculated the
257 average Procrustes shape before proceeding with further analyses; we also averaged linear
258 measurements in this manner. To visualize variation in highly dimensional shape characters, we
259 estimated principal component axes and plotted species averages in tangent space (Figure 3c and
260 e). As a proxy for body size, we used the logarithm of the centroid size of mesosoma landmarks
261 as in (Economo et al. 2015a), which behaves similarly to the Weber's Length measurement
262 typically used by myrmecologists (Weber 1938). Only multivariate Procrustes alignment data,
263 and not principle component data, were used in the comparative methods below (Uyeda et al.
264 2015).

265

266 *Phylogenetic Data*

267

268 We used a time-resolved phylogeny reconstructed by Economo et al. (2018) that includes 449
269 ingroup *Pheidole* species, based on a molecular dataset of nine loci. This phylogenetic tree builds
270 upon previous analyses of *Pheidole* (Moreau 2008; Economo et al. 2015a), with the addition of
271 164 taxa and an expanded set of loci sequenced across species. For analyses in this paper, we
272 used the maximum clade credibility tree from a Bayesian posterior set, which was pruned to
273 contain only the taxa present in our morphological data (Figure 3A).

274

275 *Comparative Methods*

276

277 To examine the degree of correlated evolution between body regions (i.e., morphological
278 integration), we used the *R* package *geomorph* (Adams and Otárola-Castillo 2013). We ran a
279 series of pairwise integration tests between body regions and worker castes (Adams and Collyer
280 2017). In each test, we estimated partial least squares (PLS) correlations between two sets of
281 landmarks (e.g., major's head and minor's head) while correcting for phylogeny. The coefficient
282 of correlation (r-PLS) for this regression describes the degree of integration. To calculate a p-
283 value and significance test, we generated 1000 permutations of species' phylogenetically-
284 transformed values for each comparison. To compare evolutionary integration of body size
285 among worker castes and between the head and mesosoma, we used the coefficient of correlation
286 for the linear regression of phylogenetically independent contrasts, hereafter r-PIC (Felsenstein
287 1985). To compare the relationship between trait shape and body size, we used a phylogenetic
288 regression implemented for Procrustes shape variables (Adams and Collyer 2018). These and
289 other methods described below were also run for the New World, Old World, and Australasian
290 clades individually (Figure 3A). It is important to note that integration may exceed the values
291 estimated here using PLS, as integration may span multiple PLS axis dimensions beyond the first
292 axis, which is what we compared.

293
294 We used *geomorph* to estimate evolutionary rates for landmarked specimens (Denton and Adams
295 2015). As a significance test for differences in rates between traits, we performed 1000
296 simulations of trait evolution under a joint Brownian motion model, and compared the ratio of
297 independently estimated rates to this simulated null. Given that differences in the number of
298 landmarks can bias the amount of variation and thus rate described by each trait (Denton and
299 Adams 2015), we report rate ratios for each pair of traits (e.g., major head vs. major mesosoma)
300 as a proportion of the simulated null ratio.

301
302 We tested for evidence of evolutionary modularity within each body region (i.e., in addition to
303 the head and mesosoma) again using *geomorph* (Adams and Otárola-Castillo 2013). We split
304 each body region into sets of a priori evolutionary modules (*sensu* Klingenberg 2008) roughly
305 aligned with anatomical axes. Head landmarks were assigned to two potential module
306 arrangements, one along the anterior/posterior axis (hereafter: A/P), and one along the
307 sagittal/lateral axis (hereafter: S/L; see Figure 4A). The A/P grouping separates the anterior

308 (clypeus) area which is related to the feeding apparatus from the posterior of the head which
309 houses the brain and mandible muscles. The D/V axis separates structures more toward the
310 midline of the head (central clypeus, antennae) from the sides (eyes, occipital lobes). Mesosoma
311 landmarks were also assigned to three potential groupings, one along the anterior/posterior axis
312 with bias towards the anterior (hereafter: A/p), one along a similar axis with bias towards the
313 posterior (a/P), and one along the dorsal/ventral axis (D/V; see Figure 4A). These
314 anterior/posterior groupings correspond to landmarks associated with different body segments,
315 while the D/V grouping associates landmarks in the region closer the legs or dorsal part of the
316 body, respectively. In this framework, we compared the covariance ratio (CR; Adams 2016) of
317 each hypothesized set of landmarks to those of simulated sets of landmarks (averaged between
318 orientations rotated up to 90° in 0.05° increments), while accounting for phylogenetic
319 relationships. Each simulation test was run for 1000 iterations.

320

321

322

323

324 **Results**

325

326 *Evolutionary Rate*

327

328 In comparisons of different body regions of the same worker caste, mesosoma shape evolved
329 more rapidly than head shape in both major workers (rate ratio $rr = 6.02$, $p < 0.01$) and minor
330 workers ($rr = 6.14$, $p < 0.01$; Figure 5). In comparisons of similar traits between worker castes,
331 we observed no significant differences in evolutionary rate for head shape ($rr = 1.07$, $p = 0.59$) or
332 mesosoma shape ($rr = 1.10$, $p = 0.59$). In contrast to the rate variation among shape traits,
333 evolutionary rates estimated for size traits showed few differences between worker castes or
334 between the head and mesosoma (Figure 6), with the exception of the major worker's head
335 which evolved relatively slowly.

336

337 Our tests of modularity within body regions suggested the presence of two evolutionary modules
338 in the *Pheidole* head, in an anterior-posterior arrangement, though the use of semi-landmarks

339 may bias this result. We compared evolutionary rate between the inferred modules of head shape
340 (Figure 4A). In these analyses, the anterior landmarks exhibited a higher rate of evolution than
341 the posterior landmarks in both major workers ($rr = 1.42$, $p < 0.05$) and minor workers ($rr = 1.42$,
342 $p < 0.001$).

343

344 *Morphological Integration*

345

346 Morphological integration is described here as correlated evolution between morphological
347 shape characters. The strength of this correlation is described using the PLS correlation
348 coefficient (r-PLS), and its significance is assessed by comparison to a simulated null
349 distribution (Adams and Felice 2014; Adams and Collyer 2016). For estimates of body size
350 rather than shape it is measured as the correlation coefficient of independent contrasts (r-PIC).

351

352 We found strong indications of morphological integration between both worker castes and body
353 regions in *Pheidole*, however the strength of these correlations varied depending on the
354 comparison (Figure 7a). Head shape was correlated with mesosoma shape in both major workers
355 ($r\text{-PLS} = 0.53$, $p < 0.001$) and minor workers ($r\text{-PLS} = 0.51$, $p < 0.001$). In examinations of
356 morphological integration between worker castes, mesosoma shape was strongly correlated
357 between castes ($r\text{-PLS} = 0.76$, $p < 0.001$), whereas head shape showed a weaker albeit still
358 significant correlation ($r\text{-PLS} = 0.48$, $p < 0.001$). This difference in worker caste integration
359 effect among body regions was highly significant (two-sample z test; $p < 0.001$). Similar results
360 were observed for analyses performed with semi-landmarks from the head's posterior lateral
361 lobes included. Morphological integration varied somewhat between clades, with the Asian-
362 African clade exhibiting a lower degree of integration for all shape traits.

363

364 We performed hierarchical clustering on correlation coefficient matrices for shape integration
365 and size integration (Figure 7b, Figure 7c). Overall, *Pheidole* showed much greater
366 morphological integration in size than in shape. Morphological integration was greater for size
367 traits ($r\text{-PIC} 0.8 - 0.95$) than for any shape traits (maximum $r\text{-PLS} = 0.76$). This integration in
368 size was greater within worker castes than between them (Figure 7c). Morphological integration
369 of shape traits was greatest between the mesosoma of major and minor workers, which evolved

370 as though it were a single module. Head shape was weakly integrated with other traits for minor
371 workers, and least integrated for major workers (Figure 7b).

372

373 The scaling relationship between the sizes of different parts is a common theme in evolution and
374 development. As expected, we found a tight relationship between mesosoma size (Weber's
375 length; Weber 1938) and head length; this was evident in both majors and minors. Relationships
376 between the shape of the head and mesosoma and body size were observable, however they were
377 very weak and poorly predictive (all R-squared values < 0.03).

378

379 *Modularity Within Body Regions*

380

381 Modularity is measured in geomorph as the covariance ratio (CR), which describes the
382 covariation between modules relative to the covariation within modules (Adams 2016). When
383 $CR \geq 1$, modules show no observable signal of modularity; values significantly less than 1
384 (compared to a simulated null distribution) indicate independence between modules. We
385 estimated modularity for two a priori configurations for head landmarks, and three a priori
386 configurations for mesosoma landmarks (Figure 4A).

387

388 We tested for modularity in head shape using two datasets: one including only fixed landmarks,
389 and one also including semi-landmarks describing the curvature of the posterior lateral lobes.
390 Using only fixed landmarks, head shape showed no significant modularity along the A/P axis in
391 major workers ($CR = 1.31$, $p = 1.00$; see also Figure 4B) or minor workers ($CR = 1.28$, $p = 1.00$).
392 We observed similarly non-significant scores when dividing landmarks into a S/L axis; this was
393 consistent across both major workers ($CR = 1.17$, $p = 0.91$) and minor workers ($CR = 1.18$, $p =$
394 0.87). However, when we included (semi-landmark) data on the posterior lateral lobes, we
395 observed that head shape evolved as two independent modules along the anterior/posterior axis
396 in major workers ($CR = 0.79$, $p = 0.003$) and in minor workers ($CR = 0.76$, $p = 0.002$). No such
397 effect was observed along the sagittal/lateral axis for either major workers ($CR = 0.98$, $p = 0.26$)
398 or minor workers ($CR = 1.05$, $p = 0.69$). For mesosoma shape, we observed no significant or
399 even weak evidence of modularity (always $CR > 1$; see Figure 4B). However, few potential

400 module configurations exist for a set of only 6 landmarks, which likely limited our ability to
401 detect modularity with this dataset.

402

403

404 **Discussion**

405

406 Our results showed varying evolutionary rates and degrees of evolutionary integration within and
407 among worker castes; thus, evolutionary rate and integration followed the predictions of different
408 hypotheses (Figure 1). In particular, the mesosoma exhibited integration among homologous
409 traits in different worker castes, while the head exhibited a weaker degree of integration. We
410 found that the mesosoma evolved faster than the head and with a greater degree of
411 morphological integration between castes (Figure 5), but in general evolutionary rate was similar
412 for homologous traits in different worker castes. We found a complex pattern whereby the
413 evolution of the head shape of major workers was largely decoupled from that of other traits, but
414 was not necessarily evolving faster.

415

416 The evolutionary rate of carapace shape was highly divergent across the different parts of the ant
417 (head vs. mesosoma). This observation was most evident with regards to mesosoma shape, which
418 evolved roughly 1.5x faster than head shape (when corrected for variance differences). Moreover,
419 we found that the anterior portion of the head near the mandibles and mouthparts is evolving
420 more quickly than the posterior half. However, there were no significant differences in
421 evolutionary rate among homologous traits between majors and minors. Thus, homologous traits,
422 and not traits within a caste, tended to evolve at similar rates (Figure 1).

423

424 The inferred rate similarity among traits does not alone imply the traits themselves are correlated
425 in their evolution (i.e. they could be evolving at similar rates but on different trajectories), thus
426 we also investigated which sets of traits were correlated during evolution. Here, we found a
427 different pattern, whereby the evolution of mesosoma shape was tightly linked across major and
428 minor workers, but head shape was more decoupled between the two castes. In this way, the
429 head of the major worker was the least integrated with other traits, and the mesosoma of the
430 worker was the most integrated. Previous research in *Pheidole* found that integration among

431 linear measurements was weaker for minor workers than major workers (Pie and Traniello 2007).
432 In contrast, our analyses found weaker integration between head and mesosoma shape for majors
433 than minors. Thus, no one integration hypothesis was supported – either between homologous
434 traits, or between traits within a caste – but rather a mixture of the two.

435
436 The fact that mesosoma shape evolved more rapidly than head shape is somewhat surprising, as
437 the head would presumably be the most related to feeding ecology, a key trait that varies across
438 ant species. One potential explanation is that head shape is under stronger stabilizing selection.
439 However, another potential conclusion is that fast mesosoma evolution reflects relative size and
440 arrangement variation in the underlying muscles that control load-carrying and locomotion,
441 which could reflect functional differences in how the ant carries, moves, and performs different
442 tasks. The primary axis of mesosoma variation runs from a stocky shape to a more gracile and
443 elongate one, and most changes are happening repeatedly within limited bounds. There is reason
444 to expect that stocky shapes are common in belowground-foraging species, and that more gracile
445 characteristics are associated with aboveground-foraging and associated defensive traits like
446 spines (Weiser and Kaspari 2006; Sarnat et al. 2017). *Pheidole* are known to vary in the extent to
447 which they live and forage in the leaf litter or on vegetation (Mertl et al. 2010), and there could
448 be tradeoffs inherent the designs adapted for moving and foraging on horizontal vs. vertical
449 surfaces. This would also explain why major and minor mesosomas are tightly integrated in
450 shape, because they face similar biomechanical challenges due to living and moving in similar
451 environments. Thus, these phenotypes may represent ecomorphs that are repeatedly evolved in
452 each newly colonized region, as in *Anolis* lizards (Mahler et al. 2013). However, given the
453 paucity of behavioral observations for most ant species around the world, further study is
454 required to understand this trait's functional and biomechanical significance. Furthermore, the
455 linking of external geometry with variation in underlying function and performance remains an
456 important avenue for future work on comparative anatomy and biomechanics in ants.

457
458 We find support for the hypothesis that the shape of minor and major worker castes can evolve to
459 some extent independently (Holley et al. 2016), promoting the evolution of ecological
460 specialization. We emphasize that this is not simply a statement that head shapes are different
461 between majors and minors, which is obvious, but that they can evolve on diverging trajectories

462 (i.e. the major is not just a consistent transformation of the minor). This allows for increased
463 evolutionary “degrees of freedom” in the functional specialization among castes. However, this
464 finding was specific to the head region, as mesosoma shape was tightly integrated across castes.
465 The fact that rates of shape evolution were 1.5 times greater for the highly integrated mesosoma
466 than for the head (Figure 5) suggests that integration in this case does not constrain, but may
467 rather accelerate rates of evolutionary divergence in shape among species (Cheverud 1995; but
468 see Márquez and Knowles 2007).

469
470 Allometry is a common theme and pattern in development and evolution, and strong
471 relationships between the sizes of different body parts are expected during evolution. Matching
472 this expectation, we found that head and mesosoma sizes were tightly linked both within and
473 among castes (Figure 7). In contrast to the pattern for cranial evolution in birds (Klingenberg and
474 Marugán-Lobón 2013), relationships between shape traits and body size were significant, but
475 poorly predictive. While we were not able to account for allometric relationships within species
476 due to our study design, we did find that cross-species relationships between body size and shape
477 traits were not strong enough to potentially drive other patterns reported in this study. Our
478 estimates of evolutionary rate for size traits showed that the size of each trait evolved faster than
479 its shape (Figure 6), confirming a similar observation by Pie and Tscha (2013). Interestingly,
480 major worker heads evolved at the slowest rate for size and among the slowest for shape despite
481 being the least integrated with other body parts (which should thus release it from constraint by
482 pleiotropic effects; but see Cheverud 1995). This suggests that this trait is more evolutionarily
483 conserved; future studies investigating the evolutionary consistency of major worker tasks (as in
484 Mertl et al. 2010) and their biomechanical needs would be valuable in explaining this pattern.

485
486 In principle, correlations in size and shape among traits/castes could be caused by either
487 selection or developmental constraint. This kind of comparative analysis does not by itself allow
488 for inference of the underlying selective or developmental mechanisms responsible for the
489 patterns of integration that we identify. However, there is a strong body of work on the
490 developmental basis of caste differentiation in *Pheidole*, and especially the role of JH as a
491 developmental switch mediator, that can inform the likelihood of some potential explanations.
492 Notably, classic (Wheeler and Nijhout 1981, 1983, 1984; Wheeler 1991) and more recent

493 (Rajakumar et al. 2012; Rajakumar et al. 2018) work shows that experimental manipulation of
494 pheromone exposure can alter the relative sizes of *Pheidole* majors and minors, and manipulation
495 of rudimentary wing discs can alter the relative sizes of the head and body (Rajakumar et al.
496 2018). Moreover, in other insects, it has been shown that relative sizes of different body parts
497 can be experimentally selected for (Frankino et al. 2005; Stillwell et al. 2016). If researchers can
498 manipulate relative size with apparent ease using chemical cues or artificial selection, this
499 implies that evolution may not be constrained from doing the same. We expect that general
500 diversification of body size is likely to be due to selection on loci that control body size overall,
501 rather than independent selection on the size of each part. However, the fact that relative sizes of
502 different parts have been maintained in evolutionary time implies selective advantages of the
503 relative sizes of body parts within and among castes (Gould 1966).

504
505 To our knowledge, less is known about the developmental basis of the shape characters we are
506 capturing in our landmark system, so developmental constraints or biases may explain some of
507 the evolutionary correlation in shape we observe. However, the evolutionary modules in the head
508 inferred by our analysis (Figure 4A) do not correspond to the head developmental modules
509 inferred by Yang and Abouheif (2011) in their examination of *Pheidole* gynandromorphs. If both
510 studies are correct, this would imply that developmental modularity does not underlie the
511 macroevolutionary modularity we infer, leaving selection and non-genetic influences, as well as
512 methodological issues with comparing fixed landmarks and semi-landmarks, as the most likely
513 explanations for why different regions of the head appear to evolve separately or independently.
514 An interesting future direction would be to attempt to experimentally investigate the
515 developmental bases of the axes of shape variation we identify in our study.

516
517 One noticeable feature of the genus *Pheidole*'s global diversification has been the re-evolution of
518 similar environmental and behavioral niches in different geographic regions, each radiation
519 following from a single colonization event (Moreau 2008; Economo et al. 2015a). While
520 morphological evolution in this clade has been largely conserved throughout its history (Pie and
521 Traniello 2007), similar body size phenotypes have consistently re-evolved following each
522 clade's colonization of a new biogeographic realm (Economo et al. 2015a). In this study we
523 observed that New World and Old World radiations of *Pheidole* occupied mostly overlapping

524 portions of morphospace (Figure 3), whereas the Australasian clade occupied a smaller, but still
525 overlapping portion of this same trait space. We found this pattern for size and shape of both
526 head and mesosoma. It remains unclear why some portions of morphospace, and large body size
527 in particular, have not evolved in Australasian taxa. One potential explanation is that niche filling
528 in this most recent radiation is ongoing – indeed the Australian clade is the youngest of the
529 continental radiations and is still in a more elevated phase of its diversification (Economo et al.
530 2019).

531

532 *Conclusion*

533 The morphological and functional differentiation of castes is thought to be a key evolutionary
534 innovation underlying the success of ants and other social insects. Patterns of macroevolutionary
535 integration and modularity within and among castes may provide clues to the selective forces
536 shaping diversification in ants, and the developmental biases and constraints involved in trait
537 divergence (West-Eberhard 1979). We find that size evolution is tightly integrated and evolving
538 with homogeneous rates both among parts in a single caste, and across the worker castes. In
539 contrast, our results using geometric morphometric estimates of body shape indicate that while
540 mesosoma shape shows homology integration, head shape has become largely disintegrated
541 between major and minor workers (Figure 3c). Head morphology and its associated musculature
542 is associated with ecological specialization in many taxa, often but not exclusively due to feeding
543 functionality, thus the differences in head shape between major and minor workers probably
544 represent divergence in their tasks in the colony (Smith 1987; Futuyma and Moreno 1988; Mertl
545 and Traniello 2009). In this case, evolution of developmental pathways facilitating independent
546 evolution of major and minor worker phenotypes could represent key innovations enabling
547 lineages with this trait to occupy multiple specialized strategies at once, or to discover new team
548 strategies emergent from their polymorphism (Wheeler & Nijhout 1981, 1984; Wheeler 1990;
549 Anderson and McShea 2001). Interestingly, the independent evolution of the head does not lead
550 to faster rates of evolution, and in fact mesosoma shape evolves 1.5x faster than head shape in
551 *Pheidole*. We hypothesize that this rapid evolution of the mesosoma reflects a pattern of frequent
552 adaptation to different biomechanical needs in different microhabitats, but future work is needed
553 to test this hypothesis.

554

555 While body-size polymorphism is a common trait in ants, “complete” polymorphism (i.e., in
556 shape) is rarer but noticeably present in some of the most diverse ant clades (Wills et al. 2017),
557 an observation that hints at a role for polymorphism in adaptability (Wilson 2003). We propose
558 that, beyond the benefits of body-size polymorphism, the reduction of morphological integration
559 between distinct behavioral strategies, inclusive of sexes, castes, and alternative reproductive
560 tactics (West-Eberhard 1979), could be a recurring key innovation that enables the evolution of
561 adaptive polymorphism and promotes rapid diversification. Further comparative studies on the
562 evolution integration and modularity across radiations of ants with worker polymorphisms, and
563 any concurrent changes in diversification rates and patterns, would be useful for testing this
564 hypothesis.

565

566 **Acknowledgements**

567 We thank R. Keller, Y. Hashimoto, C. Peeters, S. Price, A. Saurez, and members of the Economo
568 Unit for providing stimulating discussion, and A. Lazarus and M. Ogasawara for photographing
569 specimens. C. Klingenberg and several anonymous reviewers provided constructive comments
570 that contributed to this work. EPE, LLK, and JPH were supported by NSF (DEB-1145989). NRF,
571 BL, GF, EMS, and EPE were supported by subsidy funding to OIST, and EPE and NRF were
572 supported by Japan Society for the Promotion of Science KAKENHI grants (17K15180 and
573 17K15178, respectively).

574

575 **Figure Titles**

576

577 Figure 1: Hypothesized scenarios for the evolution of differentiated phenotypes. Worker castes
578 or body parts united in the same box represent a pair of integrated traits. The scenarios we
579 propose can be arranged in order of their extent of integration among homologous traits in
580 different castes and among different traits within a caste.

581

582 Figure 2: Example photographs of *fervens* minor worker (A) and major worker (B) assembled by
583 photo-montage according to AntWeb specifications. Landmarks, in white, were placed on
584 homologous features on the head (C) and mesosoma (D). Semi-landmarks, in blue, were spaced

585 equally on the left side of the head between landmarks 3 and 11, and between landmarks 11 and
586 1.

587
588 Figure 3: A phylogeny of the ant genus *Pheidole*, with clades colored by their geographic region,
589 is shown in (A). Note that each clade represents a single colonization event (see Economo et al.
590 2015a). Comparisons of values for like traits in different castes are shown for head size (B), head
591 shape (C), mesosoma size (D), and mesosoma shape (E). Ellipses reflect 95% confidence
592 intervals, and are colored according to clade as in (A). For the shape data displayed in (C) and
593 (E), the first principle component is shown for display purposes (and is not used in subsequent
594 comparative methods), along with the percentage of variance it explains and deformation grids
595 describing extreme values along the axis (produced using *geomorph*; Adams et al. 2018).

596
597 Figure 4: Hypothesized evolutionary module configurations (A) are shown with landmarks
598 assigned to each module assigned different colors. The table in (B) shows results of
599 phylogenetically-corrected modularity tests conducted in *geomorph*. Covariance Ratios (CR) are
600 given for each hypothesized configuration, as well as p-values derived from comparison against
601 a simulated null.

602
603 Figure 5: Evolutionary rates are displayed here as a morphogram heat map (Martin &
604 Wainwright 2011). Comparison ratios between traits digitized using different numbers of
605 landmarks (e.g., head and mesosoma) are given as ratios compared to a simulated null ratio.
606 Arrows and brackets indicate statistical tests of rate differences compared to a simulated null,
607 with accompanying numbers describing the estimated rate ratio for the two traits. * $p < 0.05$,
608 ** $p < 0.01$, *** $p < 0.001$

609
610 Figure 6: Comparison of evolutionary rate estimates for size and shape of *Pheidole* body parts
611 and worker castes.

612
613 Figure 7: Morphological integration between among body parts within and among worker castes
614 is shown by arrow width in (A). Hierarchical clustering of integration relationships for trait
615 shape is shown in (B) and for trait size in (C), with the strength of relationships indicated by the

616 heatmap and displayed value – r-PLS for trait shape and r-PIC for trait size. *p < 0.05, **p<0.01,
617 ***p<0.001

618

619 **Author Contributions**

620 NRF drafted the manuscript, and NRF and BLB performed the analyses. EPE, LLK and NRF
621 designed the study; BLB, GF, EMS, and NRF collected morphological data; EPE, LLK and JPH
622 sequenced specimens and constructed a phylogeny. All authors edited and approved the
623 manuscript.

624

625 **Data Accessibility**

626 Data for this paper can be accessed on Dryad at <https://doi.org/10.5061/dryad.gqnk98sjx>.

627

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629

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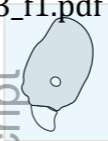
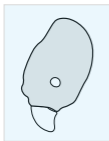
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No Integration

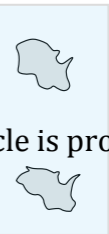
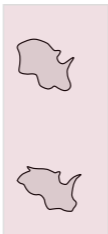
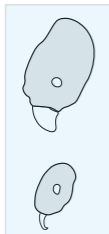
Integration within castes

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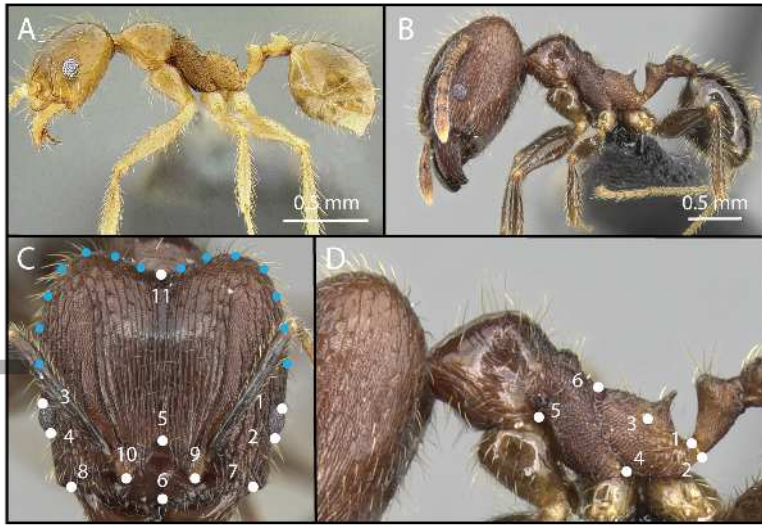
Integration among homologous traits

Complete Integration

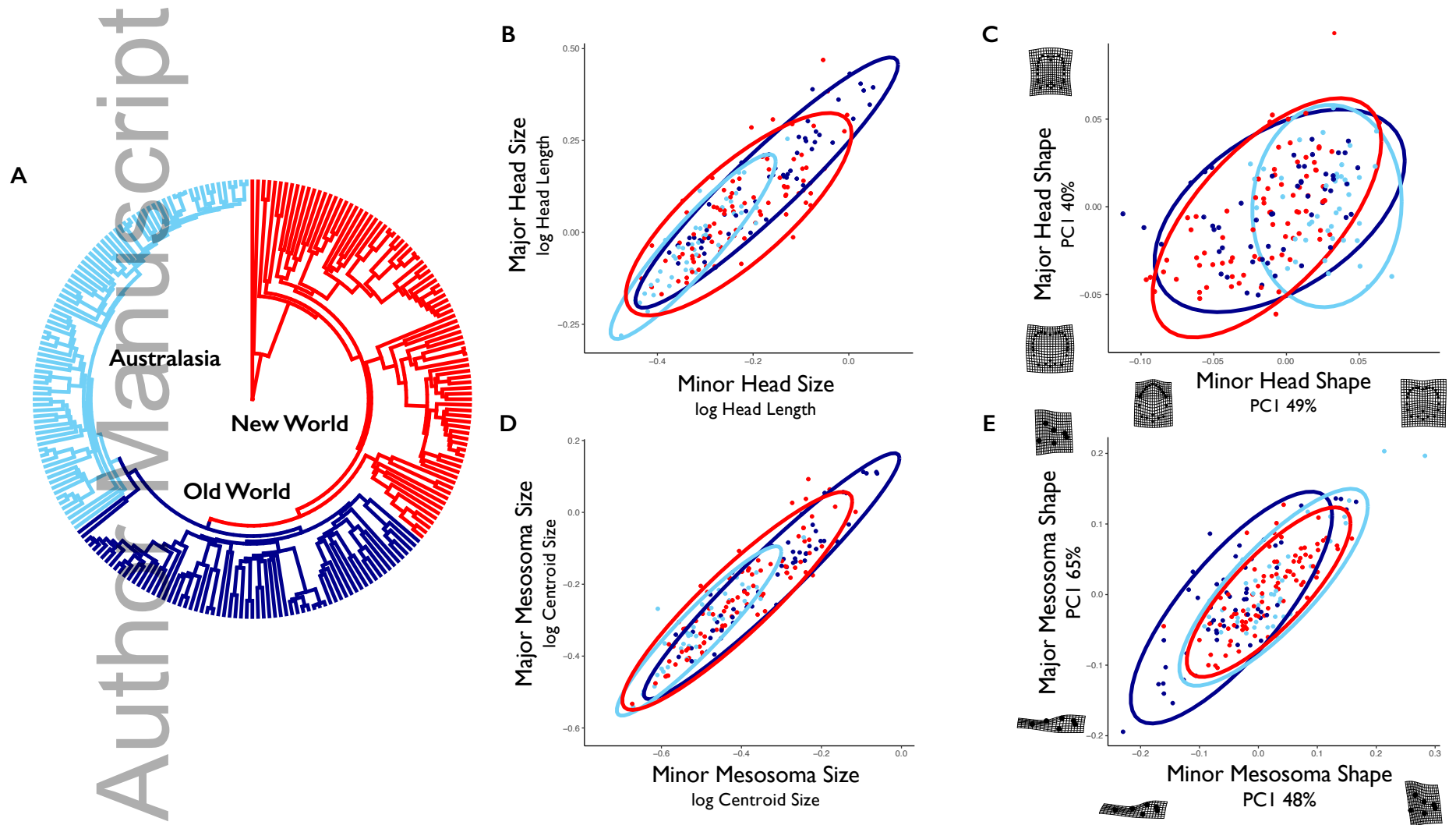


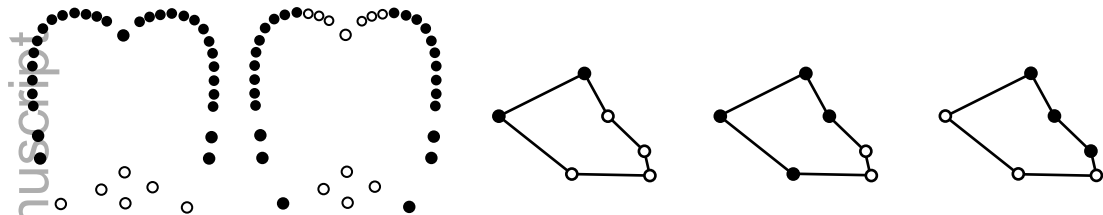
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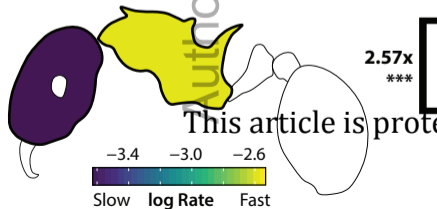
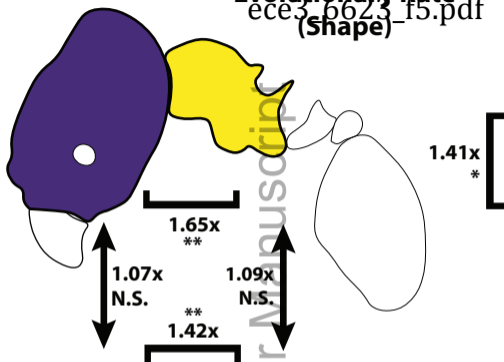


A**B**

		A/P	S/L	a/P	A/p	D/V
major	CR	0.73	0.98	2.97	2.17	1.75
	p	0.001	0.18	1.00	1.00	0.90
minor	CR	0.90	0.98	2.64	1.54	2.35
	p	0.027	0.30	1.00	0.48	1.00

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