

### Colony size predicts division of labour in Attine ants.

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1 Colony size predicts division of labour in Attine ants

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## 18 Summary

19 Division of labour is central to the ecological success of eusocial insects, yet the evolutionary  
20 factors driving increases in complexity in division of labour are little known. The size-  
21 complexity hypothesis proposes that, as larger colonies evolve, both non-reproductive and  
22 reproductive division of labour become more complex as workers and queens act to maximise  
23 inclusive fitness. Using a statistically robust phylogenetic comparative analysis of social and  
24 environmental traits of species within the ant tribe Attini, we show that colony size is  
25 positively related to both non-reproductive (worker size variation) and reproductive (queen-  
26 worker dimorphism) division of labour. The results also suggested that colony size acts on  
27 non-reproductive and reproductive division of labour in different ways. Environmental  
28 factors, including measures of variation in temperature and precipitation, had no significant  
29 effects on any division of labour measure or colony size. Overall, these results support the  
30 size-complexity hypothesis for the evolution of social complexity and division of labour in  
31 eusocial insects. Determining the evolutionary drivers of colony size may help contribute to  
32 our understanding of the evolution of social complexity.

33 Key Index words: Formicidae, queen-worker dimorphism, worker size polymorphism, social  
34 evolution, caste evolution

35

## 36 1. Introduction

37 Insect eusociality represents one of the major transitions in evolution [1-3]. In these events,  
38 groups of formerly free-living individuals become sufficiently integrated to be considered  
39 individuals in their own right. A key component of this process is the evolution of division of  
40 labour [1, 4, 5]. In eusocial societies, the presence of a sterile caste (workers) and a dedicated  
41 reproductive caste (queens) creates a reproductive division of labour, while behavioural or  
42 morphological specialization within the worker caste on tasks such as brood care, nest  
43 maintenance, foraging and defence creates a non-reproductive division of labour. In 'simple'  
44 eusocial societies, queens are morphologically similar to workers, and workers are  
45 monomorphic. In 'complex' eusocial societies, queen-worker dimorphism is extreme and there  
46 is wide variation in worker size, often accompanied by discrete physical worker castes [4, 6,  
47 7]. Previous studies have suggested positive effects of division of labour on the foraging  
48 efficiency and colony productivity of social insects, and hence on their ecological success [8-  
49 11]. However, the evolutionary determinants of division of labour have been less well  
50 researched.

51 The 'size-complexity hypothesis' proposes that, as colony size increases, workers and queens  
52 maximize their inclusive fitness by specializing in non-reproductive and reproductive roles,  
53 respectively [4, 6, 12, 13]. Since such specialization permits further increases in colony size,  
54 the degree of non-reproductive and reproductive division of labour both increase via positive  
55 feedback between social complexity and colony size. The hypothesis therefore leads to the  
56 prediction that colony size is positively associated with two key aspects of social complexity -  
57 non-reproductive and reproductive division of labour.

58 Although theoretical models [14, 15] and single taxon experimental studies [16-18] offer  
59 some support for the size-complexity hypothesis, whether the predicted across-species  
60 relationships occur remains unclear, as early comparative studies [6, 19] were informal and  
61 lacked an explicit evolutionary framework [20]. More recent phylogenetic comparative

62 studies across formicoid ant species [21] and corbiculate bees [22] found positive correlations  
63 between colony size and measures of social complexity. While informative, these studies  
64 either omitted species with very large colony sizes ( $> 10^6$  workers or more) [21], potentially  
65 missing the predicted relationships [4], or measured social complexity as a single variable  
66 [22], potentially missing the independent effects of colony size on individual components of  
67 social complexity, namely the extent of reproductive and non-reproductive division of labour  
68 [21].

69 Moreover, no previous study has considered possible effects of environmental factors, yet  
70 these also potentially influence the relationship between colony size and division of labour.  
71 For example, in ants, a non-linear relationship exists between colony size and primary  
72 productivity such that higher primary productivities are associated with decreasing colony  
73 size [23]. This suggests that it is important to control for environmental factors when  
74 analysing correlates of colony size across species. Environmental factors may also influence  
75 division of labour directly. Experiments show that in the desert ant *Cataglyphis velox*, smaller  
76 workers forage at lower temperatures than larger ones, suggesting that worker size variation  
77 has evolved as a mechanism for colonies to cope with diurnal fluctuations in temperature  
78 [24]. Overall, therefore, the potential role of environmental factors needs to be considered to  
79 gain a full understanding of the evolution of division of labour.

80 To test for evolutionary relationships between division of labour, colony size and  
81 environmental factors, we gathered species-specific data on social traits and evolutionary  
82 relationships and conducted a phylogenetically-controlled comparative analysis within the  
83 neotropical ant tribe Attini. We used worker size variation and queen-worker dimorphism as  
84 measures of non-reproductive and reproductive division of labour, respectively. We selected  
85 ants as the focal taxon because ants are the most socially diverse and ecologically successful  
86 social insect group [7, 25]. We focused on the tribe Attini because this taxon (252 species)  
87 exhibits wide variation in worker size, queen-worker dimorphism and colony size, and occurs  
88 in a relatively broad range of habitats and latitudes [26-32]. In addition, the Attini are

89 predominantly monogynous [26], i.e., having a single queen heading a colony, such that the  
90 size-complexity hypothesis can be tested in the absence of confounding effects of variation in  
91 colony genetic and social structure brought about by polygyny (multiple queens heading  
92 colonies) [4, 33]. Controlling for environmental variation, we show that evolutionary  
93 increases in colony size across the Attini are associated with increases in both worker size  
94 variation and queen-worker dimorphism.

95

## 96 2. Materials and Methods

### 97 (a) Data collection

98 We used all Attini genus names, including synonyms, as search terms in Web of Knowledge,  
99 Scopus and Google Scholar literature databases up to 2013. Literature sources resulting from  
100 this search were scanned manually and relevant data were extracted. Data from secondary  
101 sources were excluded. Additional data were collected from AntWeb  
102 (<http://www.antweb.org>). Data from 58 sources covering 632 observations of populations for  
103 57 out of a total of 252 species in the Attini were collected (see electronic supplementary  
104 material, table S1). These data represented all Attine genera (except for the socially parasitic  
105 *Pseudoatta*, a derived form of *Acromyrmex* [34], and the recently erected genus  
106 *Paramyrcetophylax*). Taxonomic names followed the Bolton World Catalogue  
107 (<http://www.antweb.org>).

108 Data were collected and stored in a database following recommendations in Kattge *et al.* [35].  
109 The following traits were recorded: worker and queen size measured as head width in mm (92  
110 observations of populations for 36 and 39 species for worker and queen head widths,  
111 respectively), colony size (number of workers at colony maturity) (178 observations, 43  
112 species) and geographical location (362 observations, 48 species). Where specific coordinates  
113 were not supplied in the source reference, they were inferred from the description of the  
114 locality except where the specified area exceeded 20 km<sup>2</sup>. In these cases the locality was

115 deemed to be uninformative and excluded from analysis. Head-width measurements taken  
116 from AntWeb (<http://www.antweb.org>) (17 and 13 species for worker and queen head widths,  
117 respectively) were measured using the image analysis software ImageJ [36]. To ensure the  
118 measurements obtained from the specimens on AntWeb were representative, we compared  
119 the measurements obtained from images of seven species well represented both in the  
120 literature and on AntWeb. In all cases the AntWeb measurements were not significantly  
121 different from those obtained from the literature (paired *t*-test,  $t=1.044$ ,  $p=0.34$ ,  $n=7$ ).

122 We calculated per-species means for colony size and worker and queen head width (see  
123 electronic supplementary material, table S1) by averaging the mean value from each  
124 observation weighted by its sample size as:

$$125 \quad \bar{x} \text{ mean trait value} = \frac{\sum(x_s n_s)}{\sum n_s}$$

126 where  $x_s$  is the mean of the observation,  $n_s$  is the observation sample size, and  $\sum n_s$  is the sum  
127 of all sample sizes of the observations contributing to the per-species mean for each trait.

128 Observation sample sizes ranged from 1-1016; however, in many cases, observation sample  
129 sizes were not given in the original source and here we assumed it to equal 1. We report  $\sum n_s$   
130 as the sample size for each per-species mean trait value as this is more appropriate to the  
131 nature of our data than the number of sources.

132 To measure non-reproductive division of labour for each species, we quantified worker size  
133 variation using the coefficient of variation in worker head width (36 species) following  
134 previous authors [21]. We selected the coefficient of variation as it was an objective measure  
135 of trait variation that avoided subjective assessment of the number of discrete worker castes.  
136 In addition, using number of worker castes to measure worker size variation would not  
137 quantify non-reproductive division of labour correctly in species with size-based polyethism  
138 and a continuous distribution of worker sizes [8, 9]. Worker size variation was calculated as:

139 
$$\text{Worker size variation} = 100 \left( \frac{\sigma \text{ worker head width}}{\bar{x} \text{ worker head width}} \right)$$

140 where  $\bar{x}$  = mean,  $\sigma$  = standard deviation. Standard deviation of worker head width was  
 141 calculated as the standard deviation of all mean worker head width observations contributing  
 142 to each per-species value, and  $\bar{x}$  worker head width was calculated by averaging the mean  
 143 value from each observation weighted by its sample size. Our measure of worker size  
 144 variation was not influenced by sample sizes: a linear regression model (for data where  
 145 observation sample sizes were known, controlling for study effort) of square root worker size  
 146 variation and  $\log \Sigma n_s$  was not significant ( $\log \Sigma n_s$ , beta = 0.002, df = 2, 30,  $p = 0.857$ ).

147 To measure reproductive division of labour for each species, we quantified queen-worker  
 148 dimorphism as the percentage difference between mean queen head width and mean worker  
 149 head width (30 species), i.e. as:

150 
$$\text{Queen - worker dimorphism} = 100 \left( \frac{2(\bar{x} \text{ queen head width} - \bar{x} \text{ worker head width})}{\bar{x} \text{ queen head width} + \bar{x} \text{ worker head width}} \right)$$

151 For both measures we selected head width as a measure of body size because it is the most  
 152 commonly reported measure of queen and worker size in the literature and, although showing  
 153 allometric variation in some cases (e.g. *Atta* [37]), it correlates well with body size [7, 38-40].

154 To quantify environmental variation we downloaded the following data layers from the online  
 155 database BioClim (<http://www.worldclim.org/bioclim>): diurnal temperature range,  
 156 isothermality, temperature seasonality, and precipitation seasonality. We resampled BioClim  
 157 data from its original resolution into a grid size of 10 arc-minutes per pixel (approximately  
 158 20km<sup>2</sup> at the equator) to reflect the threshold at which we discarded locality information. The  
 159 R package “raster” [41] was used to extract these environmental values for sets of coordinates  
 160 derived from the source references for each ant species and mean values for each species were  
 161 calculated for use in subsequent analyses (48 species). Species locations ranged from latitudes



162 between 41.0° (DEC) and -29.7° (DEC), showing a broad range of environmental variation  
163 (see electronic supplementary material, table S1, figure S1).

164 (b) Phylogenetic reconstruction

165 Analyses of traits across species are often confounded by non-independence because closely  
166 related taxa have similar traits due to shared evolutionary history [42]. This non-  
167 independence can be statistically controlled for in analyses by incorporating an estimate of  
168 evolutionary relatedness. However, constructing rigorous and unbiased estimates of  
169 evolutionary relationships for all the taxa of interest is challenging when existing  
170 phylogenetic studies are incomplete and conflicting and use non-overlapping datasets [43].  
171 Previous phylogenetic analyses of social traits in ants have not used formal methods to link  
172 separate phylogenies or cover missing taxa [21, 44, 45], resulting in phylogenetic estimates  
173 that may be biased and that contain no estimates of uncertainty.

174 Here, we go beyond previous studies and construct an Attini consensus phylogeny that  
175 analyses the available phylogenetic hypotheses to generate a new, unbiased estimate,  
176 accompanied by calculations of uncertainty. We constructed a phylogeny using supertree  
177 protocols [43, 46, 47], since these methods allowed us to produce a tree that maximised the  
178 number of species in the resulting phylogeny and therefore the phylogenetic overlap with the  
179 species in our trait dataset. Available phylogenetic information for Attini is mainly based on  
180 morphological characters and is not well represented by genetic sequences in GenBank. As  
181 other consensus phylogenetic methods rely on constructing an estimate from genetic  
182 sequences (e.g. the supermatrix approach [48]), we chose the supertree method as the most  
183 appropriate for these data as it can combine both morphological and genetic evidence. We  
184 used matrix representation with parsimony (MRP) [43, 46]. This method involves coding the  
185 topologies of published phylogenies into a weighted character matrix that is analysed using  
186 maximum parsimony to produce a composite tree [49]. MRP was selected for consistency  
187 with previous studies employing supertree methods [46, 50] and has been shown to return

188 trees as well supported as those derived using other methods [51-53]. Prior to analysis we  
189 implemented safe taxonomic reduction [54] to remove species that had little or no  
190 phylogenetic signal, which if retained would reduce the resolution of the final tree. The final  
191 matrix had 71 out of 252 species drawn from 12 source phylogenies (see the electronic  
192 supplementary material, table S2), representing all genera of Attini (except for  
193 *Paramyctophylax*). We implemented a parsimony ratchet [55] in PAUP\* v.4.0b10 [56] to  
194 analyse the matrix, and took the resulting consensus. Support values for each node of the tree  
195 were generated using rQS [57], which prunes the supertree and each source tree to confer  
196 identical taxon sets on them and then compares the topologies, assigning each node a score  
197 between +1 (full support) and -1 (total conflict). Positive rQS values indicate support for a  
198 node. We obtained, aligned and concatenated 4321 bp of sequence data for five genes  
199 (*wingless*, *long-wavelength Rhodopsin*, *elongation factor 1 alpha 1*, *elongation factor 1 alpha*  
200 *2* and *cytochrome oxidase subunit 1*) from species of the Attini represented in GenBank [58].  
201 We used the software packages BEAST [59] in conjunction with the alignment to calculate  
202 relative branching time estimates for the species shared between the alignment and the  
203 supertree following previous studies [46] under a strict molecular clock [60]. Three Attini  
204 fossils were used as calibration points at nodes 11 [61], 50 [62] and 54 [63] and a non-Attini  
205 fossil (*Pheidole*) [64] was used to date node 1 (see electronic supplementary material, figure  
206 S2), allowing dates to be calculated from relative branch lengths. The perl script  
207 `chronographer.pl` [65] was used to infer missing node ages based on a pure-birth model  
208 resulting in a supertree topology with branching time estimates following [46]. The final  
209 supertree was deposited in TreeBASE  
210 (<http://purl.org/phylo/treebase/phyloids/study/TB2:S14540>).

### 211 (c) Data analysis

212 We tested all social traits for phylogenetic signal using the phylogenetic generalised least  
213 squares (PGLS) function of the R package "caper" [66]. All traits contained phylogenetic  
214 signal (worker size variation  $\lambda = 0.97$ , queen-worker dimorphism  $\lambda = 0.94$  and

215 colony size  $\lambda = 0.91$ ), and so we used phylogenetically-controlled regression models in  
216 subsequent analyses.

217 Data were checked for normality and outliers. We used a square-root transformation for  
218 worker size variation and a natural log. transformation for queen-worker dimorphism and  
219 colony size to normalize the data. We checked for collinearity in all models separately by  
220 calculating variance inflation factors (VIF) for each covariate. Covariates were sequentially  
221 eliminated starting with the largest VIF until all VIFs were less than three [67].

222 Before fitting any models we removed species from the analysis with any missing data,  
223 resulting in a dataset of 19 species. We adopted an information-theoretic approach to analyse  
224 the effects of social and environmental factors on non-reproductive and reproductive division  
225 of labour. PGLS models describing each possible iteration of specific hypotheses were fitted  
226 to the data. We used the corrected Akaike information criterion (AICc) to assess model fit,  
227 and calculated small-sample AICc weight and  $\Delta\text{AICc}$  (the difference in AICc between the  
228 model in question and the best fitting model) for each model. Models with  $\Delta\text{AICc} < 7$  were  
229 considered uninformative and were discarded [68]. Since no model had an AICc weight  $> 0.44$   
230 and the informative models for each hypothesis included between them all covariates, we do  
231 not report a single best model. We instead report relative importance and averaged parameter  
232 estimates from the set of informative models [68].

233 The averaged models were based on a single consensus phylogenetic tree (a strict consensus  
234 of 10,000 equally parsimonious trees). Parameter estimates of the models are influenced by the  
235 phylogenetic estimate used and therefore are sensitive to other reconstructions [69]. To  
236 investigate the effects of phylogenetic uncertainty on our analysis, we fitted PGLS models on  
237 a dated sample of 1,000 of the 10,000 most parsimonious trees from the PAUP\* analysis of  
238 the MRP matrix. We selected only variables that had a cumulative AICc weight of  $> 0.4$  for  
239 these models. This allowed more accurate measurements of parameter estimates, which were

240 generated as means from the sample of models, and of 95% phylogenetic uncertainty intervals  
241 [69].

242

### 243 3. Results

244 Mean worker size variation ranged from 0.23 to 64.37 (36 species), queen-worker  
245 dimorphism from 1.54% to 84.25% (30 species) and colony size from 16 to  $6 \times 10^6$  workers  
246 (43 species). The largest values for all these traits were found in the genera *Atta* and  
247 *Acromyrmex* (the leafcutter ants) (figure 1).

#### 248 (a) Phylogenetic reconstruction.

249 The topology of our supertree (electronic supplementary material, figure S2) was broadly in  
250 agreement with the most recent molecular phylogeny for the Attini [70]. Clades that emerged  
251 as paraphyletic were *Cyphomyrmex* (with respect to *Mycetophylax conformis*) (node 7) and  
252 *Trachymyrmex* (with respect to *Sericomyrmex*) (node 12). None of these relationships are  
253 novel [70-72] and no novel clades were generated [73]. Furthermore, the supertree recovered  
254 the three clades of Attini defined by the nature of their fungal-agricultural system, i.e. the  
255 lower Attines (which cultivate environmentally derived fungi), the higher Attines excluding  
256 leafcutters (which engage in obligate fungal symbiosis but do not harvest fresh leaves) and  
257 the leafcutters (which engage in obligate fungal symbiosis and harvest fresh leaves) [31, 70].  
258 The mean rQS score over 10000 bootstrap replicates of the tree was 0.282 and only three  
259 (nodes 52, 59 and 60) of the 60 nodes had a negative rQS score (reflecting more mismatches  
260 than matches in the source trees) (see electronic supplementary material, table S3). We dated  
261 the root node (node 1) to 37.7 million years (MY) ago, the node representing the origin of the  
262 higher Attini to 17.3 MY ago and the origin of the leafcutters to 12 MY ago. While this root  
263 estimate is 8.3-17.3 MY younger than equivalent nodes on other molecular trees [70], the  
264 other values of the other nodes are within the confidence intervals of previous estimates [70].

265 (b) Determinants of non-reproductive division of labour

266 Colony size was significantly positively correlated with worker size variation (table 1; figure  
267 2). All models featured colony size as a covariate and had a range of high  $R^2$  values (0.770-  
268 0.818), and colony size had a cumulative AICc weight of 1, showing its importance in all  
269 supported models. Furthermore, colony size was the only covariate in the averaged model to  
270 have confidence intervals that did not include zero (table 1). The presence of queen-worker  
271 dimorphism, mean diurnal temperature range and precipitation seasonality in the averaged  
272 model suggest they have an effect on worker size variation; however, all three of these  
273 covariates had confidence intervals that included zero (table 1). Models omitting colony size  
274 had no support ( $w_i=0$  in both cases, electronic supplementary material, table S4a). These  
275 models were robust to phylogenetic uncertainty (table 1). Differences in mating systems  
276 among the Attini could have potentially confounded our analyses as species that were found  
277 to exhibit the largest colony sizes and worker size variation (leafcutter ants) are polyandrous  
278 [72]. To investigate this, we reanalysed our data including mating system as a dichotomous  
279 variable (0, monandrous and 1, polyandrous) in the models. We used all data on the presence  
280 and absence of polyandry from the literature and, for non-leafcutter ant species where data  
281 were not available, we assumed monandry (electronic supplementary material, table S1). We  
282 found the significance of the correlation between colony size and worker size variation to be  
283 unchanged whilst controlling for queen-worker dimorphism and mating system (for colony  
284 size,  $\beta = 0.271$  (CI = 0.133, 0.509),  $W = 0.93$ , results from an averaged model).

285 (c) Determinants of reproductive division of labour.

286 To complement the analysis of Fjerdingstad & Crozier [21], which found that colony size and  
287 worker size variation across 35 ant species were not significantly associated after controlling  
288 for queen-worker dimorphism, we first ran a model that included worker size variation as a  
289 covariate. This found no effect of colony size on queen-worker dimorphism. The resulting  
290 averaged model had only parameter estimates with confidence intervals that included zero

291 (table 1). The best fitting model set also captured less of the variation in queen-worker  
292 dimorphism than the models for worker size variation ( $r^2 = 0.031-0.342$ ). These analyses were  
293 robust to phylogenetic uncertainty (table 1). However, according to our VIF threshold (VIF  
294 for worker size variation = 4.80), colony size and worker size variation could not be in the  
295 model together. We therefore ran models omitting worker size variation, which showed  
296 colony size to be a positive predictor of queen-worker dimorphism (table 1). The effect was  
297 not as powerful as the effect of colony size on worker size variation, and the covariate was  
298 not universally shared in the most informative models (cumulative AICc weight = 0.85).  
299 Overall, therefore, we found a significant positive correlation between colony size and queen-  
300 worker dimorphism, but this result was weaker than the correlation of colony size with  
301 worker size variation. Moreover, it disappeared when worker size variation was included as a  
302 covariate, either because of shared variance or because worker size variation predicts queen-  
303 worker dimorphism better than colony size.

304 (d) Environmental determinants of colony size.

305 We found no significant correlations between colony size and any of the environmental  
306 variables tested (table 1). The  $r^2$  value of all models was low (range 0.001-0.211) and in all  
307 resulting average models the confidence intervals of the covariates overlapped with zero.

308

#### 309 4. Discussion

310 In agreement with the size-complexity hypothesis [4, 6, 12, 13], our study shows that colony  
311 size is significantly positively correlated with measures of non-reproductive and reproductive  
312 division of labour in a tribe of ants. These findings provide novel support for the size-  
313 complexity hypothesis; we detected a strong relationship between colony size and worker size  
314 variation independent of the effects of queen-worker dimorphism, we controlled for  
315 environmental factors and we separated social complexity into component traits. Our results  
316 are also consistent with a recent study linking colony size with another predicted correlate of

317 social complexity [4, 6, 12], namely divergence in queen and worker lifespans in the eusocial  
318 Hymenoptera [44]. In addition, our results strengthen the idea that group size and complexity  
319 are positively related in the evolution of other levels of complexity within the hierarchy of  
320 major transitions, such as the evolution of multicellularity [4, 5, 13, 74].

321 We found no evidence for any effects of environmental factors on worker size variation,  
322 queen-worker dimorphism or colony size. Although colony size and primary productivity  
323 appear to be associated in ants, the relationship is non-linear [23] and, in general,  
324 relationships between colony size, latitude and climatic variables vary considerably across ant  
325 taxa [75]. Therefore, the lack of effects of environmental factors in our study could have  
326 arisen because Attini are exceptions to the colony size-primary productivity relationship or  
327 because the study sampled species across the range of primary productivities where the  
328 relationship is approximately flat [23].

329 Our results suggest that colony size acts upon the two forms of division labour in different  
330 ways. Specifically, we found that the positive association between colony size and queen-  
331 worker dimorphism became non-significant when worker size variation was included,  
332 whereas the positive association between colony size and worker size variation remained  
333 significant in both the presence and absence of queen-worker dimorphism. If the two forms of  
334 division of labour responded to increasing colony size in the same way, we would have  
335 expected to see any combination of the two measures result in the absence of a positive  
336 association (due to very high colinearity). One plausible scenario that could account for our  
337 findings is non-simultaneous evolution of the two traits. A potential mechanism for this arises  
338 from an assumption of the size-complexity hypothesis, namely that the chance of any given  
339 worker attaining direct fitness falls as colonies evolve to become larger [4, 6, 12]. If so, this  
340 would lead workers' inclusive fitness interests to coincide more closely with those of queens  
341 at larger colonies sizes [4], since workers would be more strongly selected to maximise their  
342 fitness indirectly by aiding the direct reproduction of queens. Selection for worker size  
343 variation, which helps improve colony productivity [9, 76], might then lead to even stronger

344 selection for increased fecundity in queens and hence to greater queen-worker dimorphism.

345 This hypothesis could be tested by investigating the order of trait divergence among worker

346 size variation, queen-worker dimorphism and colony size, or by investigating the rates of

347 evolutionary change of these traits.

348 An unexpected association from our results was a link between fungal-agricultural system and

349 colony size. This was shown by the clustering of the three agricultural groups within the

350 Attini, i.e., lower Attines, higher Attines (excluding leafcutter ants) and leafcutter ants, within

351 the continuum of association between worker size variation and colony size (figure 2). To

352 investigate this more formally, we examine the relationship between colony size and fungal-

353 agricultural system. We find that colony size has a highly significant effect on agricultural

354 system when treated as either a continuous variable (PGLS,  $\beta=0.12$ ,  $p < 0.001$ ), or a

355 categorical variable (univariate multinomial logistic regression, see electronic supplementary

356 material, multinomial model analysis). Although it is not possible from current data to

357 determine the evolutionary sequence of events, a possible scenario is that shifts in the fungal-

358 agricultural system in the Attini act as ecological drivers permitting increases in colony size,

359 and that these then lead to increases in the complexity of division of labour proposed by the

360 size-complexity hypothesis and detected by our analysis.

361 As phylogenetic reconstructions and large datasets of social and environmental trait data

362 become increasingly available, studies like the present one that combine the power of

363 phylogenetically-controlled analyses with the rich social and ecological diversity of eusocial

364 insects will help test the size-complexity hypothesis in additional taxa and, more generally,

365 investigate further how social and environmental factors influence the evolution of social

366 complexity and division of labour within societies.

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Table 1. Averaged models describing effects of covariates on worker size variation, queen-worker dimorphism (where (a) and (b) represent models excluding and including worker size variation, respectively) and colony size in the Attini. Regression coefficients and confidence intervals are reported from best ( $\Delta\text{AICc} > 4$ ) PGLS models from full candidate sets (see electronic supplementary material, tables S4a, b and c). Bold type indicates significant covariates.

Covariates	Worker size variation	Queen-worker dimorphism (a)	Queen-worker dimorphism (b)	Colony size
(intercept)	$\beta=1.806$ (-0.554, 4.165), $pCI\pm 0.000$	$\beta=1.659$ (1.644, 1.675), $pCI\pm 0.031$	$\beta=0.923$ (-0.957, 2.802), $pCI\pm 0.010$	$\beta=4.230$ (-3.954-12.414), $pCI\pm 0.146$
Colony size	<b><math>\beta=0.392</math> (0.227, 0.559), <math>pCI\pm 0.000</math>, <math>W=1.00</math></b>	<b><math>\beta=0.159</math> (0.042, 0.276), <math>pCI\pm 0.001</math>, <math>W=0.85</math></b>	$\beta=0.135$ (-0.030, 0.301), $pCI\pm 0.005$ , $W=0.60$	-
Worker size variation	-	-	$\beta=0.235$ (-0.127, 0.598), $pCI\pm 0.011$ , $W=0.52$	-
Queen-worker dimorphism	$\beta=0.108$ (-0.497, 0.713), $pCI=n/a$ , $W=0.48$	-	-	-
Mean diurnal temperature range	$\beta=-0.015$ (-0.035, 0.0058), $pCI\pm 0.000$ , $W=0.69$	-	-	$\beta=-0.021$ (-0.071, 0.029), $pCI\pm 0.001$ , $W=0.40$
Isothermality	-	$\beta=-0.004$ (-0.044, 0.036), $W=0.28$	$\beta=-0.003$ (-0.046, 0.038), $W=0.33$	$\beta=0.022$ (-0.126, 0.170), $pCI\pm 0.001$ , $W=0.50$
Temperature seasonality	-	-	-	-
Precipitation seasonality	$\beta=0.013$ (-0.017, 0.043), $pCI\pm 0.000$ , $W=0.041$	$\beta=0.0009$ (-0.014, 0.016), $W=0.28$	$\beta=0.004$ (-0.009, 0.016), $W=0.36$	$\beta=-0.015$ (-0.210, 0.179), $pCI\pm 0.001$ , $W=0.62$
Isothermality*precipitation seasonality	-	-	-	$\beta=0.003$ (-0.0002, 0.006), $W=0.13$
Latitude	-	-	-	$\beta=0.009$ (-0.077, 0.096), $W=0.19$

Key:  $\beta$  = model averaged regression slope (95% confidence intervals),  $pCI$  = 95% confidence interval for the regression slope from 1000 models including parameters with  $W > 0.4$  from a sample of 1000 equally likely trees;  $W$  = cumulative AICc weight over all models from the full candidate model set.

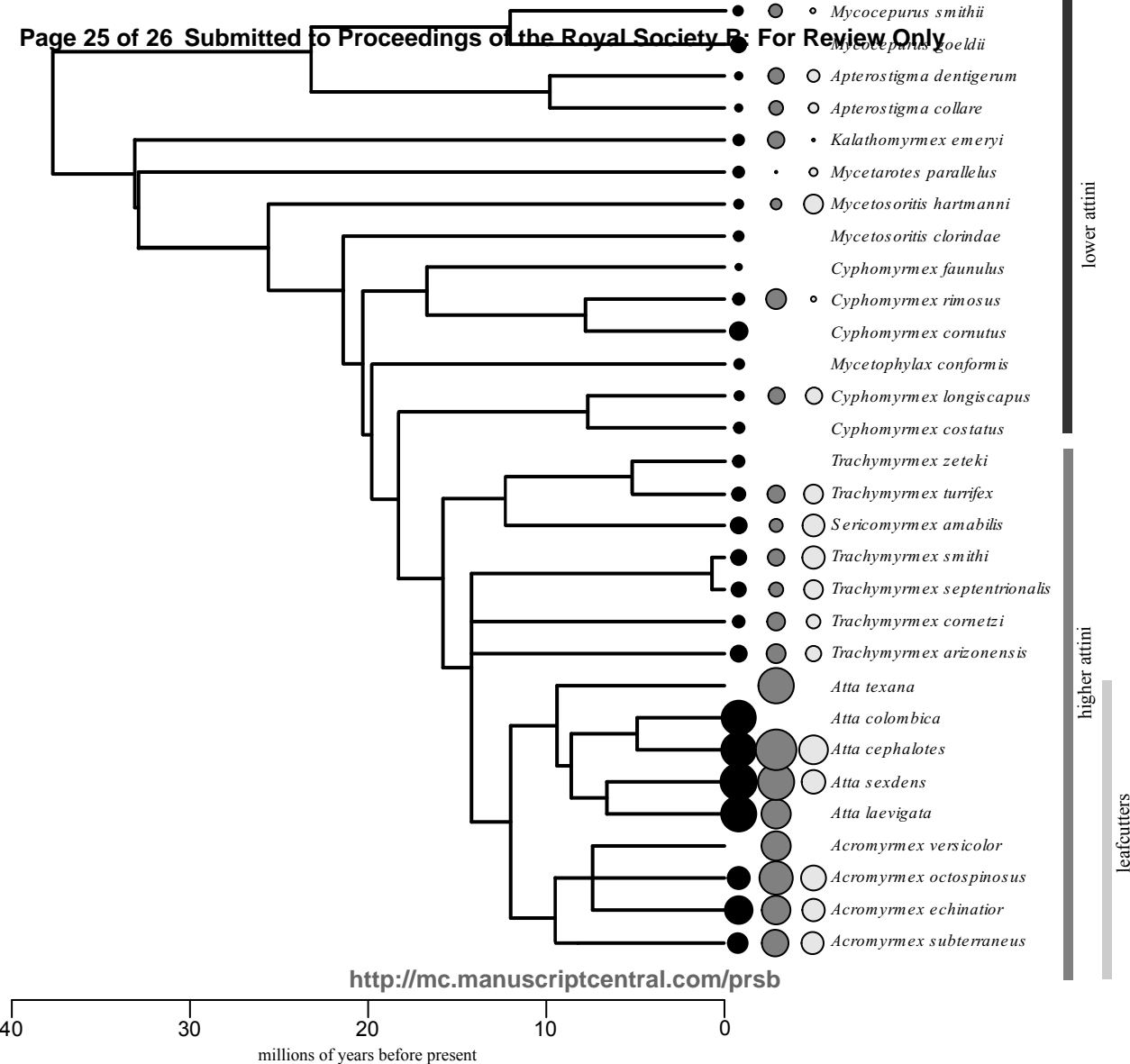


548 Figure legends

549 Figure 1. Distribution of colony size, worker size variation and queen-worker dimorphism on  
550 a phylogenetic supertree for the Attini (30 species). The full tree (electronic supplementary  
551 material, figure S2) was pruned to include only the species for which there were data on at  
552 least one trait and appeared in the phylogeny. Black circles are proportional to  $\ln$  mean colony  
553 size, grey circles to the square root of worker size variation and white circles to  $\ln$  queen-  
554 worker dimorphism. Branch lengths are proportional to time (millions of years).

555

556 Figure 2. The relationship between  $\ln$  mean colony size and square root worker size variation  
557 in the 19 species of Attini for which colony size and worker-size variation data were  
558 available; triangles represent the lower Attini, circles the higher Attini (excluding the  
559 leafcutter ants) and squares the leafcutter ants. Slope and intercept are taken from the  
560 phylogenetically controlled averaged model (table 1), and dotted lines are  $\pm$  95% confidence  
561 intervals from the same model.



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40 30 20 10 0  
millions of years before present

lower attini

higher attini

leafcutters

