

1 **TITLE PAGE**

2 **Title:** Modularity and connectivity of nest structure scale with colony size

3

4

5 **Running head:** Nest architecture scales with colony size

6

7

8 **Authors:** Julie S. Miller¹, Emma Wan¹, Sean O'Fallon¹ & Noa Pinter-Wollman¹

9

10

11 **Author Affiliations**

12 1. Department of Ecology and Evolutionary Biology, University of California Los Angeles,
13 612 Charles E. Young Dr. East, Los Angeles, CA 90095-7246.

14

15

16 **Corresponding Author:** julie.serena@gmail.com; +1-310-766-3581; 612 Charles E. Young Dr.
17 East, Los Angeles, CA 90095-7246.

18

19 **Author contributions:** JSM conceived of the study, analyzed the data and wrote the first draft of
20 the paper. JSM and EW collected the data. SO collected data on ant body lengths. JSM and NPW
21 designed the study, and all authors helped write the manuscript.

22

23 **Acknowledgements:** We are thankful to Doug Booher for sharing his nest castings and to
24 Walter Tschinkel for additional images of his published castings. We thank Dana Williams,
25 Felipe Zapata, Kevin Loope, Tyler McCraney and the UCLA IDRE statistical consulting unit for
26 advice on the statistical analysis and to Saray Shai for advice on generating reference models.
27 We thank Gabby Najm, James Lichtenstein, Natalie Lemanski, Andrea Perna, and two
28 anonymous reviewers for helpful comments on the manuscript. The authors declare no conflicts
29 of interest.

30

31

32 **Funding:** JSM was supported by NSF Postdoctoral Research Fellowship in Biology DBI
33 1812292 and NSF grants IOS 1456010/1708455 to NPW.

34

35

36 **Data Accessibility Statement:** Data available on Dryad, DOI <https://doi.org/10.5068/D18D7B>;
37 Code available on Zenodo, DOI <https://doi.org/10.5281/zenodo.5636719>

38 **TITLE:** Modularity and connectivity of nest structure scale with colony size

39

40 **ABSTRACT**

41

42 Large body sizes have evolved structures to facilitate resource transport. Like unitary organisms,
43 social insect colonies must transport information and resources. Colonies with more individuals
44 may experience transport challenges similar to large-bodied organisms. In ant colonies, transport
45 occurs in the nest, which may consist of structures that facilitate movement. We examine three
46 attributes of nests that might have evolved to mitigate transport challenges related to colony size:
47 (1) subdivision - nests of species with large colonies are more subdivided to reduce crowd
48 viscosity; (2) branching - nest tunnels increase branching in species with large colonies to reduce
49 travel distances; and (3) short-cuts – nests of species with large colonies have cross-linking
50 tunnels to connect distant parts of the nest and create alternative routes. We test these hypotheses
51 by comparing nest structures of species with different colony sizes in phylogenetically controlled
52 meta-analyses. Our findings support the hypothesis that nest subdivision and branching evolved
53 to mitigate transport challenges related to colony size. Nests of species with large colonies
54 contain more chambers and branching tunnels. The similarity in how ant nests and bodies of
55 unitary organisms have evolved in response to increasing size suggests common solutions across
56 taxa and levels of biological organization.

57

58 **KEYWORDS:** nest, morphological evolution, network, colony size, ants, allometry

59

60 INTRODUCTION

61 The consequences of body size for the evolution of phenotypic diversity are far reaching,
62 influencing morphological (Schmidt-Nielson 1984), physiological (Kleiber 1947), and
63 behavioral traits (Calder 1984; Dial et al. 2008) in systematic ways (Peters 1983). Evolutionary
64 allometry (Thompson 1917; Huxley 1932), the study of how body size relates to trait size across
65 lineages (Gould 1971; Cheverud 1982), has offered a window into the underlying mechanisms
66 that drive trait diversity (e.g. McMahon 1975; Fairbairn 1997; Frankino et al. 2005). An
67 important consequence of increasing body size is that it alters the basic physical conditions in an
68 organism by increasing the distances over which transport of nutrients, waste products, and
69 signals must traverse (Bonner 2004). This physical transport challenge has been resolved through
70 the evolution of a variety of structural features across the tree of life (McMahon and Bonner
71 1983), including multi-cellularity (Niklas and Newman 2016), body plans that increase surface-
72 area-to-volume ratios (e.g., jellyfish and sponges; Ruppert et al. 2004), branching vascular
73 systems in animals and plants (West et al. 1997; Banavar et al. 1999), and compartmentalization
74 of specialized tissues into organs (Gould 1966).

75 Social insect colonies are often compared to whole organisms (Wheeler 1910; Seeley
76 1989; Hölldobler and Wilson 2009), and large colonies may experience transport challenges that
77 are similar to those of large bodied organisms (Gillooly et al. 2010). In larger colonies, the speed
78 and/or reliability with which resources, or information, reach disparate parts of the colony will
79 depend on transfer occurring among more individuals and across greater distances (Pacala et al.
80 1996; O'Donnell and Bulova 2007; Dornhaus et al. 2012). The outstanding diversity of the ant
81 lineage (Hölldobler and Wilson 1990), and multiple independent evolutionary increases in

82 colony size (Burchill and Moreau 2016), provide an ideal foundation for comparative work that
83 investigates how physical structures evolved to mitigate transport challenges related to size.

84 Transport of resources and information in social insect colonies may depend on the
85 layout of their nests. Nest structure affects the routes that individuals take as they move resources
86 and information. Therefore, nest structure influences the way in which individuals interact
87 (Pinter-Wollman et al. 2013; Pinter-Wollman 2015b; Tschinkel 2015; Pinter-wollman et al.
88 2018; Vaes et al. 2020) and behave as a collective (Burd et al. 2010; Pinter-Wollman 2015a).
89 Having more individuals may slow the delivery of resources and information, but the physical
90 structure of the nest can promote rapid movement. Here we investigate macroevolutionary trends
91 in how key nest traits, including subdivision, branching, and short-cuts, relate to typical colony
92 size, i.e., the average number of individuals in a colony, in ants (Formicidae).

93 Subdivision, or compartmentalization, is one structural feature that large systems evolved
94 to mitigate the challenges of size. For example, large multicellular organisms consist of
95 subdivided cells and organs (Kempes et al. 2012; Okie 2013). However, the size of the units are
96 constrained by the costs of expanding surface area-to-volume ratios (Bonner 1988; Lorenz et al.
97 2011). In social insect nests, larger nest chambers can hold more individuals, but make it harder
98 for individuals to contact each other (Brian 1956; Aguilar et al. 2018). Constraining the size of
99 nest chambers reduces the number of individuals occupying each chamber while reducing the
100 distances they need to travel to interact. The “subdivision hypothesis” predicts that the nests of
101 species with larger colony sizes will consist of more chambers compared to nests of species with
102 small colonies, while chamber sizes will not differ in relation to colony size.

103 Another way that large systems have evolved to improve transport is by increasing their
104 connectivity and in turn, reducing travel distances. Travel distances can be reduced by adding

105 paths that branch off from main routes. Many natural systems exhibit branching structures which
106 reduce travel distances without the addition of cross-linking paths. For example, hierarchical
107 branching structures are common in the vascular networks of animals (West et al. 1997; Tekin et
108 al. 2016) and plants (McKown et al. 2010; Blonder et al. 2018). Branching structures are
109 common when resources enter through a single site and need to be homogenously distributed
110 throughout the system (Dodds 2010). The “branching hypothesis” predicts that nests of species
111 with larger colonies will have tunnels with high branching frequency to reduce travel distances in
112 the nest.

113 Reducing travel distances and increasing connectivity can further be accomplished by
114 adding cross-linking paths, or short-cuts, that connect distant parts of a network and create
115 alternative access routes to maintain traffic flow (Corson 2010; brains: Bullmore and Sporns
116 2012; plants: Katifori et al. 2010; Blonder et al. 2018; termite nests: Valverde et al. 2009). The
117 “short-cut hypothesis” predicts that nests of species with larger colonies will contain more cross-
118 linking paths than nests of species with smaller colonies. Despite the transport benefits of adding
119 short-cuts, every additional connection has a building and maintenance cost, and highly
120 connected nests may be structurally unsound (Monaenkova et al. 2015).

121 Ants offer an ideal system to study how nest structure co-evolves with group size on
122 macroevolutionary scales because ants span a large range of colony sizes (Bourke and Franks
123 1995), and exhibit tremendous structural diversity in their nests (Tschinkel 2015). Early lineages
124 of ant species have small to medium sized colonies (Burchill and Moreau 2016) and build small
125 nests with a single tunnel and/or chambers (Tschinkel 2003), similar to their Apoid wasp
126 ancestors (Branstetter et al. 2017). The evolutionary radiation of the ants resulted in a diversity
127 of nest structures and forms, but their diversity has not been quantified and related to patterns of

128 colony size evolution. Furthermore, it is unknown how the transport challenges introduced by
129 large colony sizes have been mitigated in the evolutionary history of ants. Here, we focus on
130 ground-nesting species because, out of all ants, they have the greatest potential to control the
131 structure of their nests.

132 Prior studies of subterranean ant nest structure in relation to colony size have focused on
133 the ontogeny of single species, showing that ant nests grow in volume and increase chamber size
134 and/or number as colonies age (Tschinkel 1987, 2004, 2005, 2011, 2015). Fewer studies have
135 examined how tunnel connectivity changes with colony development (Buhl et al. 2004a), so
136 branching patterns in ant nests are not well understood. Furthermore, these studies on the
137 ontogeny of nest structure do not provide information on the evolution of nest structures in
138 relation to mature colony sizes, which we examine here in a phylogenetically controlled cross-
139 species comparison.

140 Here we investigate interspecific variation in ant nest structure and colony size within a
141 phylogenetic context. We consider an adaptive hypothesis for the evolution of nest structure
142 diversity: if ant colonies face size-associated challenges in transport and communication,
143 analogous to other systems, then the structure of nests should relate to colony size in ways that
144 ease the movement of individuals, resources, and information throughout the nest. We quantify
145 three attributes of nest structure, (1) subdivision, (2) branching, and (3) short-cuts, and examine
146 whether these traits relate to colony size in phylogenetically controlled, cross-species meta-
147 analyses. We further consider that variation in nest structure could be the result of non-adaptive
148 processes, like genetic drift or constraints imposed by evolutionary history. To determine the
149 extent to which phylogenetic history predicts variation in nest structure, we measure the
150 phylogenetic signal for the above nest traits and colony size.

151

152 **METHODS**

153 **Obtaining nest and colony size data**

154 To test our hypotheses, we gathered data on nest architecture of subterranean ant species. We
155 identified publications with graphical representations of ant nests by searching for the terms
156 “nest”, “structure”, “architecture”, “casting”, and “excavation/ed” on Google Scholar and Web
157 of Science. We identified 24 papers that contained photographs or illustrations of castings or
158 excavations of complete ant nests with distinguishable entrances, tunnels, and chambers. For the
159 subdivision hypothesis, we included data from 3 additional papers which contain tables with
160 measures of chamber size and number, even though nest images were not available (Table S1).
161 We excluded 14 publications from our dataset because interior chambers and/or tunnels were
162 obscured, nests were constructed under artificial conditions, or were from incipient colonies
163 (Table S2). Authors were contacted for additional images when parts of the nest were obscured,
164 accounting for approximately 10% of the images we used. Physical castings of unpublished nests
165 were also obtained (Table S1). When more than one nest was available for a species, we took an
166 average of all measurable nests.

167 To relate nest structure with colony size – i.e., the number of ants in a colony - we
168 searched the scientific literature and the web (AntWiki.org and AntWeb.org) for information on
169 the number of workers in a mature colony. For the majority of species, we used the average
170 colony size from the literature. When only a range (minimum - maximum) for number of ants
171 was available, we took the midpoint. Information about the number of ants in a colony at the
172 time of nest excavation or casting was available in less than half of the studies we obtained nest
173 structures from, but when available, we used the reported number of adult workers for those
174 particular nests. When colony size was not available for a particular species, or when nest

175 identity at the species level was unknown, we used colony sizes representative of the order of
176 magnitude for the genus obtained from a sister species. This approximation was necessary for the
177 *Forelius sp* nest cast in Anza Borrego, CA (Booher, personal communication). At this site, only
178 two species of *Forelius* occur and they have the same reported colony size, so we used the
179 colony size from one of them - *F. pruinosus* (10^4). For *Dorymyrmex bureni* we used the only
180 species from this genus with a recorded colony size - *Dorymyrmex bicornis* (10^3) (Table S1).
181 We excluded *Pheidole oxyops* and *Acromyrmex subterraneus* nests from our analysis because
182 colony size was not available for these species, and because we could not make a reliable
183 approximation due to substantial variation in recorded colony sizes (Table S2).

184 Nests in our sample come from species belonging to 6 subfamilies, including from the
185 most diverse subfamilies (Myrmecinae, Formicinae, and Dolichoderinae), and the early
186 branching Ponerinae, which tend to be characterized by smaller colony sizes and more basal traits
187 in general. However, we lacked nest data from other early lineages, like the Leptanillinae and the
188 Amblyoponinae.

189

190 **Nest Subdivision Hypothesis**

191 To test the subdivision hypothesis, we related colony size to the number and size of nest
192 chambers. We counted chambers in 296 nests from 43 species in 24 genera and measured their
193 sizes in 188 nests from 37 species in 21 genera. To quantify the size of chambers, we measured
194 the maximum width of each chamber using ImageJ (Rueden et al. 2017). We excluded nests
195 from the size analysis if images did not include a scale bar or if species identity was unknown for
196 a nest (e.g., *Forelius sp.*). We used chamber width as a proxy for chamber area because we relied
197 on 2D images of 3D structures. When we had access to physical castings, we measured chamber

198 width directly from the structures using a measuring-tape. We validated that chamber width was
199 a reliable indicator of chamber area using an independent dataset (see *Chamber Width Validation*
200 section of the Supplemental Materials).

201 To allow for cross-species comparison, we standardized chamber width to worker size.
202 Worker size was measured using a standard metric of ant body size, Weber's length (Brown
203 1953), using ant profile images containing a scale bar from AntWeb.org or other publications.
204 For all species, we measured the available images up to 8 specimens (1-8). For monomorphic
205 species and for species with continuous worker polymorphism, we took the average length from
206 all available images. For species with discrete worker polymorphism (i.e., majors and minors),
207 we used a weighted average, based on typical frequency of majors according to the literature
208 (Brown and Traniello 1998; Murdock and Tschinkel 2015). Standardized widths of all chambers
209 in each nest were averaged to obtain a single standardized chamber width per nest.

210

211 **Nest Connectivity**

212 *Nests as Networks*

213 To examine nest connectivity, we depicted each nest as a network, as in (Buhl et al. 2004b; Perna
214 et al. 2008; Viana et al. 2013; Pinter-Wollman 2015a; Kwapich et al. 2018), by manually
215 assigning a node ID to each chamber, nest entrance, tunnel junction, and ends of tunnels that did
216 not reach a chamber ('end nodes') and connecting these nodes with edges depicting tunnels. See
217 Figure S1 for an illustration of this method. When quantifying nest casts as networks we
218 excluded the "mushroom top" where the casting material pools around the nest entrance when it
219 is poured (Tschinkel 2010). We designated the nest entrance as the narrowest section of the
220 mushroom top. We obtained networks of 170 nests of 38 species from 21 genera, excluding

221 images in which the network structure could not be inferred because tunnel connections were
222 obscured or ambiguous (Table S1 & 2). We excluded polydomous nests because it was not
223 possible to calculate the network measures we used, mean travel distances and number cycles
224 (see below), without information about the structure of trails connecting different nests.

225

226 *Branching Hypothesis*

227 To test the if branching reduces travel distances in large colonies, i.e., the ‘branching hypothesis’
228 we quantified the length of the travel paths in a nest as the mean distance of a network. Mean
229 distance of a network is the sum of the lengths of the shortest paths that connect all possible pairs
230 of nodes, divided by the number of all possible node pairs (Figure 1):

$$\text{Mean Distance} = \frac{1}{n(n-1)} \sum_{i=1}^n d_{ij}$$

231 where n is the number of nodes, and d is the shortest path between all node pairs i and j
232 (Newman 2018). We used the “mean_distance()” function in igraph R package (Csárdi 2020).

233 To determine if observed nests had more branching than expected based on their size, i.e.,
234 the number of chambers, or nodes, in the network, we compared the mean distance of networks
235 from observed nests to mean distance of reference models of networks with known branching
236 properties. We evaluated how mean distance relates to nest size under different assumptions
237 about how new nodes are added to a network (i.e., generative models (Hobson et al. 2021)). We
238 used three generative reference models that represent the upper and lower bounds on
239 connectivity for how nest networks might increase in size (Buhl et al. 2004b; Bebbler et al. 2007;
240 Tero et al. 2010; Latty et al. 2011): (1) chain networks (Figure 1A), in which new nodes are
241 added to the last node in a chain – i.e., no branching; (2) triangulated networks (Figure 1C), in
242 which new nodes are connected to at least two other nodes such that they form a triangle while

243 avoiding edge overlap. We generated random triangulated networks using Delaunay
244 triangulation with the `deldir()` function in the ‘`deldir`’ R package (Turner 2020). The x,y
245 coordinates of the nodes to connect were randomly generated using the ‘`sample()`’ function; and
246 (3) minimum spanning trees (MSTs; Figure 1B), in which each new node is added to a terminal
247 node to form a new branch in a hierarchical structure. New nodes are added such that the total
248 length of the network is minimized and no cycles (Figure 1C) are formed. We generated MST
249 networks by pruning the triangulated graphs generated in (2) using the ‘`mst`’ function in the
250 ‘`igraph`’ R package (Csárdi 2020). Each of the above generative processes was run for network
251 sizes ranging from 3 to 100 nodes, in increments of one node. We repeated this process 1000
252 times for each network size for both triangulated networks and MSTs, because they were
253 probabilistic generative processes, but not for the chain network generation because each size has
254 only one solution. For each generated network, we calculated the mean distance to create
255 reference distributions for comparison with the observed data (Hobson et al. 2021; Figure 3B).
256 See R code in the supplementary materials.

257 We further scaled the observed networks to the reference models to relate mean distance
258 to colony size (number of individuals) in a way that accounted for expected changes in network
259 size (number of nodes). To create scaled measures of mean distance, we divided the observed
260 mean distance by the mean value of mean distance of the reference networks (from the 1000
261 simulations) for each network size (3-100 nodes). The closer the scaled mean distance value is to
262 1, the more similar the observed network is to the reference model. Scaled values greater than 1
263 indicate that the observed network has a higher mean distance than expected according to the
264 reference model. Likewise, scaled values less than 1 indicate a smaller mean distance than

265 expected according to the reference model. We related these scaled values with colony size using
266 phylogenetically controlled comparisons as described below in Statistical Analyses (Figure 3C).

267

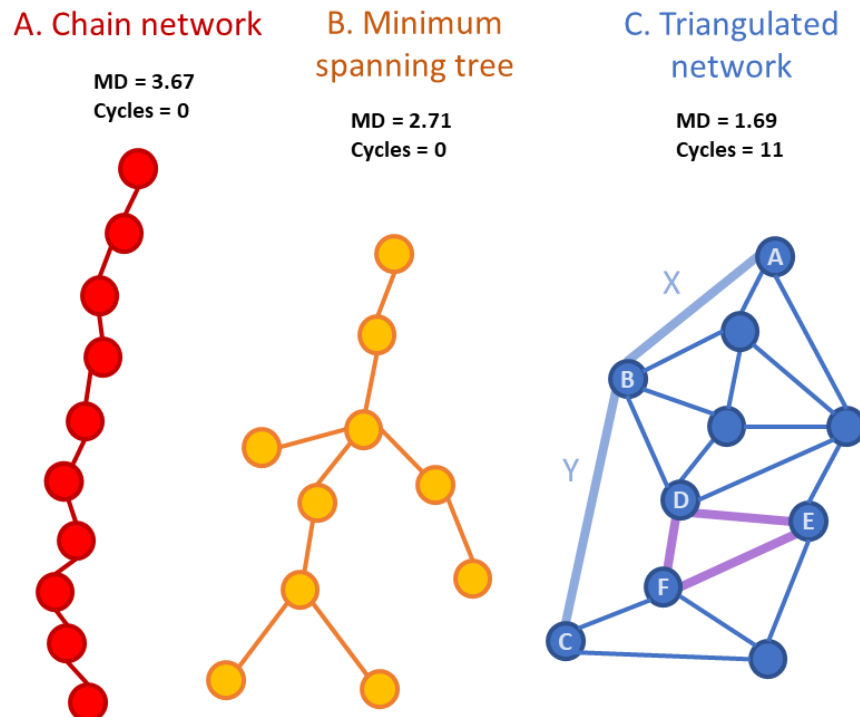
268 *Short-Cut Hypothesis*

269 To test if larger colonies have nests with more short-cuts, i.e., the short-cut hypothesis, we
270 quantified short cuts using the number of cycles in a network (Buhl et al. 2004b; Pinter-Wollman
271 2015a). A cycle is a path along a network that returns to its starting node after passing through at
272 least one other node (Newman 2018; Figure 1C). The number of cycles in a nest is:

$$\text{Number of cycles} = m - n + 1$$

273 where n is the number of nodes and m is the number of edges. Cycles are a good approximation
274 of path redundancy of a network, or presence of short-cuts, indicating how often there is more
275 than one route to any given part of the nest (Perna and Latty 2014).

276 To account for how network size affects the rate at which new cycles are added, we
277 compared the observed nests to the reference models of networks described above. Following a
278 similar procedure to the one described for network mean distance, we calculated the number of
279 cycles in triangulated networks (Figure 3E) and then scaled the number of cycles in observed
280 nests to triangulated networks of equivalent size. Because both chain and MST reference models
281 do not produce cycles, we did not include these models when we scaled the observed nests. We
282 related the scaled number of cycles in a nest with colony size using phylogenetically controlled
283 comparisons as described below (Figure 3F).



284

285 **Figure 1:** Examples of nest topologies with 10 chambers connected based on the three
286 generative reference models we tested: (A) Chain network, (B) Minimum Spanning Tree, and
287 (C) Fully connected Triangulated network. Above each network we provide its mean distance
288 (MD), which is the average of shortest paths that connect all possible pairs of nodes. As an
289 example, in (C), the length of the shortest path between nodes A and B is one, indicated by the
290 light blue edge X. The length of shortest path between nodes A and C is two, indicated by the
291 light blue edges X and Y. We further provide the number of cycles in each network. As an
292 example, in (C) the purple edges are a cycle connecting nodes D, E, and F.

293

294 **Statistical Analyses**

295 We examined the effect of colony size on the above nest measures while controlling for
296 phylogeny. We used a pruned evolutionary tree of the ants (Formicidae) from (Blanchard and

297 Moreau 2017) and calculated the mean trait value for each species. A complete species-level tree
298 was not available for all species in our dataset, so we selected only one species per genus and ran
299 our analysis using a genus-level tree (Figure 4). When there were data for more than one species
300 per genus, we used the species which had the most nests in our dataset (subset 1 in Tables S3-
301 S13). To ensure this sub-setting of species did not bias our results, we re-ran each analysis with
302 previously excluded species as representatives of a genus, and report the proportion of those runs
303 that were statistically significant. See supplementary materials for results from all runs (Tables
304 S3-13).

305 We ran phylogenetic generalized least squares (PGLS) comparing each nest trait to the
306 log of colony size. PGLS is a multiple regression that controls for the degree of similarity
307 between species based on phylogenetic distance in a co-variance matrix. We ran PGLS by
308 estimating the phylogenetic signal, lambda λ , through maximum likelihood in the “caper”
309 package v1.0.1 in R (Orme et al. 2018), with $\lambda=0$ meaning no phylogenetic signal, and $\lambda=1$
310 meaning the observations completely match phylogeny (Pagel 1999). Due to the small sample
311 size (20 - 24 genera) we could not estimate lambda with high confidence (Freckleton 2009).
312 Therefore, we further conducted PGLS for fixed lambda values (0, 1) using the “ape” package
313 v5.1 in R (Paradis et al. 2018). When our models did not meet the assumption of normality of
314 residuals we analyzed these relationships by calculating phylogenetic independent contrasts
315 (PICs) instead (Paradis et al. 2018). PIC transformed data were modeled using OLS regression,
316 with the intercept set to the origin (R Core Team 2019). Due to small sample sizes, we calculated
317 95% confidence intervals for OLS model coefficients using a bootstrap (1000 iterations).

318 To estimate phylogenetic signal for all our measures of nest structure and the log of
319 colony size, we estimated Pagel’s lambda with the ‘phylosig’ function in the “phytools” R

320 package (Revell 2017). This function estimates the value of Pagel's lambda from 1000
321 simulations and compares it to a model of no phylogenetic signal ($\lambda=0$) using a likelihood ratio
322 test. Due to small sample size, we also compared the fit of our estimated lambda model of
323 phylogenetic signal to a Brownian Motion model ($\lambda=1$) and to a model with no phylogenetic
324 signal ($\lambda=0$) using AICc values calculated from the 'fitContinuous' function in the "geiger"
325 package in R (Harmon et al. 2020). Finally, to visualize colony size and nest trait variation
326 across the phylogeny, we mapped standardized trait values onto a genus level phylogeny from
327 species subset 1 (Figure 4) using the 'contMap' and 'phylo.heatmap' functions in the "phytools"
328 R package (Revell 2017).

329

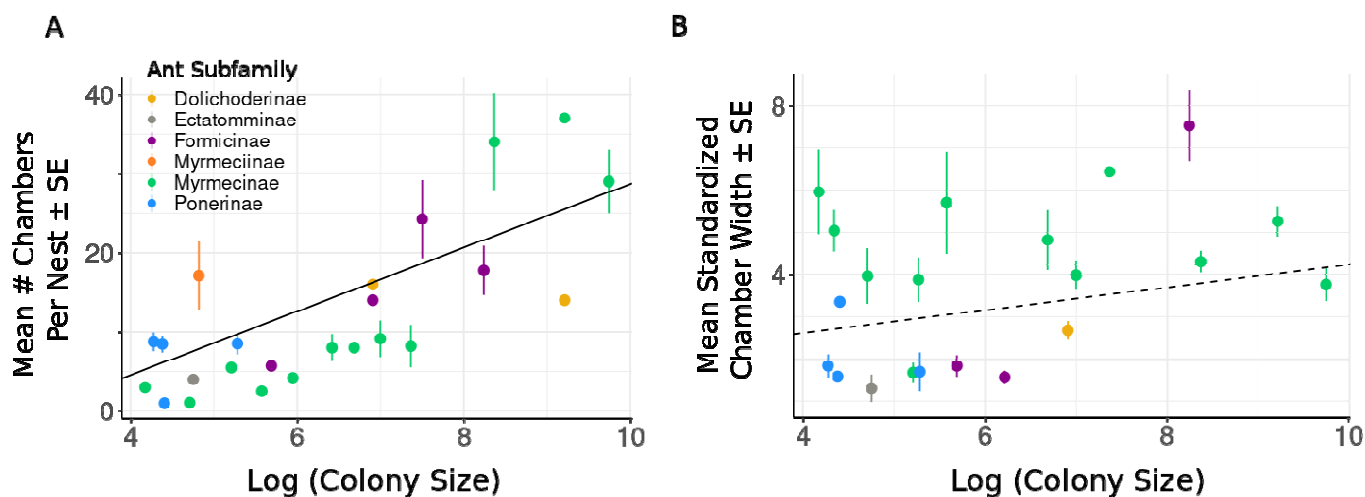
330 **RESULTS**

331

332 **Subdivision Hypothesis**

333 Both predictions of the subdivision hypothesis, that chamber number would be larger in species
334 with large colony sizes and that chamber size would not relate to colony size, were supported.
335 We found a statistically significant positive relationship between number of chambers per nest
336 and colony size (Figure 2A; N= 24 genera, OLS of PIC: $\beta = 4.024$, 95% CI = 2.014, 6.292, $R^2 =$
337 0.560, $p < 0.0001$; Table S3). The effect size of this relationship was large ($3.7 < \beta < 4.3$ across
338 all data subsets) and statistically significant in all species subsets (19/19 datasets). We did not
339 detect a statistically significant relationship between the average standardized chamber width and
340 colony size (Figure 2B; N=21 genera, OLS of PICs: $\beta = 0.350$, 95% CI = -0.049, 0.959, $R^2 =$
341 0.140, $p = 0.095$; Table S4) in the majority of the species subsets (13/15 datasets).

342



343
344 **Figure 2:** Relationship between the log of colony size (number of ants in a colony) and (A)
345 average number of chambers per nest or (B) average standardized chamber width. Each data
346 point represents a mean value for a single species, whiskers indicate standard errors when
347 multiple nests were sampled from the species. Colors represent ant subfamilies. Lines are fits
348 from the PGLS ($\lambda=1$) with a statistically significant fit as a solid line and a relationship that is not
349 statistically significant as a dashed line.

350 351 **Branching Hypothesis**

352 The “branching hypothesis”, that structural branching would reduce travel distances in nests of
353 species with large colonies, was supported. Raw, unscaled, mean distance of nests significantly
354 increased with colony size (Figure 3A; $N = 21$ genera, PGLS $\lambda=\text{MaxLL}$: $\beta = 1.36 \pm 0.247$,
355 $\lambda=0.585$, $R^2 = 0.615$, $p = 0.0007$; $\lambda=1$: $\beta = 0.844 \pm 0.227$, $p=0.0015$; $\lambda=0$: $\beta = 1.013 \pm 0.251$,
356 $p<0.0001$; Tables S5-S7). In the chain network reference models, mean distances increased
357 fastest and linearly with network size (i.e., number of nodes; Figure 3B). In the triangulated
358 reference models, mean distances were smallest and increased nonlinearly, slowing their rate of
359 increase with increasing network size. In the MST reference models, mean distances were

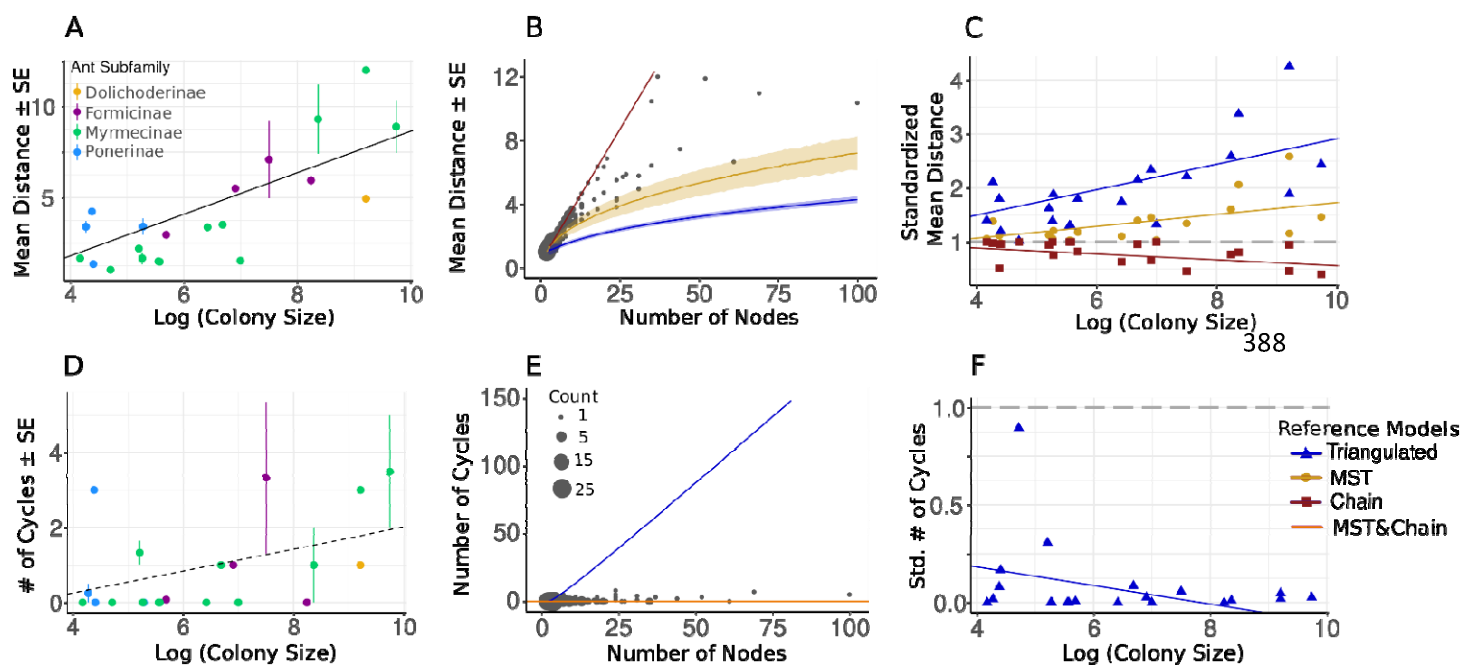
360 intermediate between the values expected from the chain and triangulated reference models, and
361 also increased nonlinearly (Figure 3B). The mean distances of empirically observed nests were
362 between the expected values for chain and MST reference models (Figure 3B). When we scaled
363 the observed mean distance with the expected mean distance based on each of the three reference
364 models, we found that the relationship between scaled mean distance and colony size differed
365 across the three models (Figure 3C). Mean distances scaled to chain networks declined with
366 colony size and the relationship was statistically significant in almost all data subsets (15/16
367 datasets; PGLS $\lambda=1$: $\beta = -0.055 \pm 0.024$, $p = 0.031$; 16/16 datasets; $\lambda=0$: $\beta = -0.061 \pm 0.024$, $p =$
368 0.018 ; Tables S8 & S9). In contrast, mean distances scaled to triangulated networks increased
369 significantly with colony size in almost all the data subsets (15/16 datasets; OLS of PIC: $\beta =$
370 0.236 , 95% CI = 0.055, 0.466, $R^2 = 0.348$, $p = 0.0048$; Table S10). Mean distances scaled to
371 MSTs also increased significantly with colony size (15/16 species subsets), but the effect size
372 was smaller than for triangulated networks (OLS of PIC: $\beta = 0.11$, 95% CI = -0.022, 0.240, $R^2 =$
373 0.281 , $p = 0.013$; Table S11).

374

375 **Short-cut Hypothesis**

376 The “short-cut hypothesis,” that nests from species with larger colonies would contain more
377 cycles, was not supported. The relationship between the number of cycles in a nest had a positive
378 relationship with colony size, but this trend was not statistically significant in the majority of the
379 data subsets (Figure 3D; 15/16 subsets; $N = 21$ genera, OLS of PIC: $\beta = 0.293$, 95% CI = -0.089,
380 0.528 , $R^2 = 0.160$, $p = 0.072$; Table S12). The triangulated reference networks showed a steep
381 increase in the number of cycles as a function of network size, and far exceeded the number of
382 cycles in observed nests given their networks’ size (Figure 3E). However, the number of cycles

383 in observed nests was similar to that expected from the chain and MST reference networks,
 384 which do not produce cycles at any network size (Figure 3E). The measure of cycles scaled to
 385 the triangulated reference models significantly decreased with colony size in all species subsets
 386 (16/16 subsets; Figure 3F; OLS of PIC: $\beta = -0.048$, 95% CI = -0.102, -0.015, $R^2 = 0.090$, $p =$
 387 0.186; Tables S13).



389 **Figure 3: Nest Connectivity: branching (A-C) and short-cut (D-F) hypotheses.** (A, D)
 390 Relationship between log colony size (average number of ants in a colony) and (A) network
 391 mean distance and (D) number of cycles in nests. Each data point represents a mean value for a
 392 single species, whiskers indicate standard errors around the mean when multiple nests were
 393 sampled for a given species, colors represent ant subfamily (see legend in A). Dashed line is the
 394 regression fit for PGLS when $\lambda=1$, solid when statistically significant and dashed when not
 395 statistically significant; (B, E) The mean distance (B) or number of cycles (E) according to
 396 network size (number of nodes). Networks from observed nests are plotted as grey dots, sized
 397 according to sample size. In (B) lines are mean distances as a function of number of nodes for

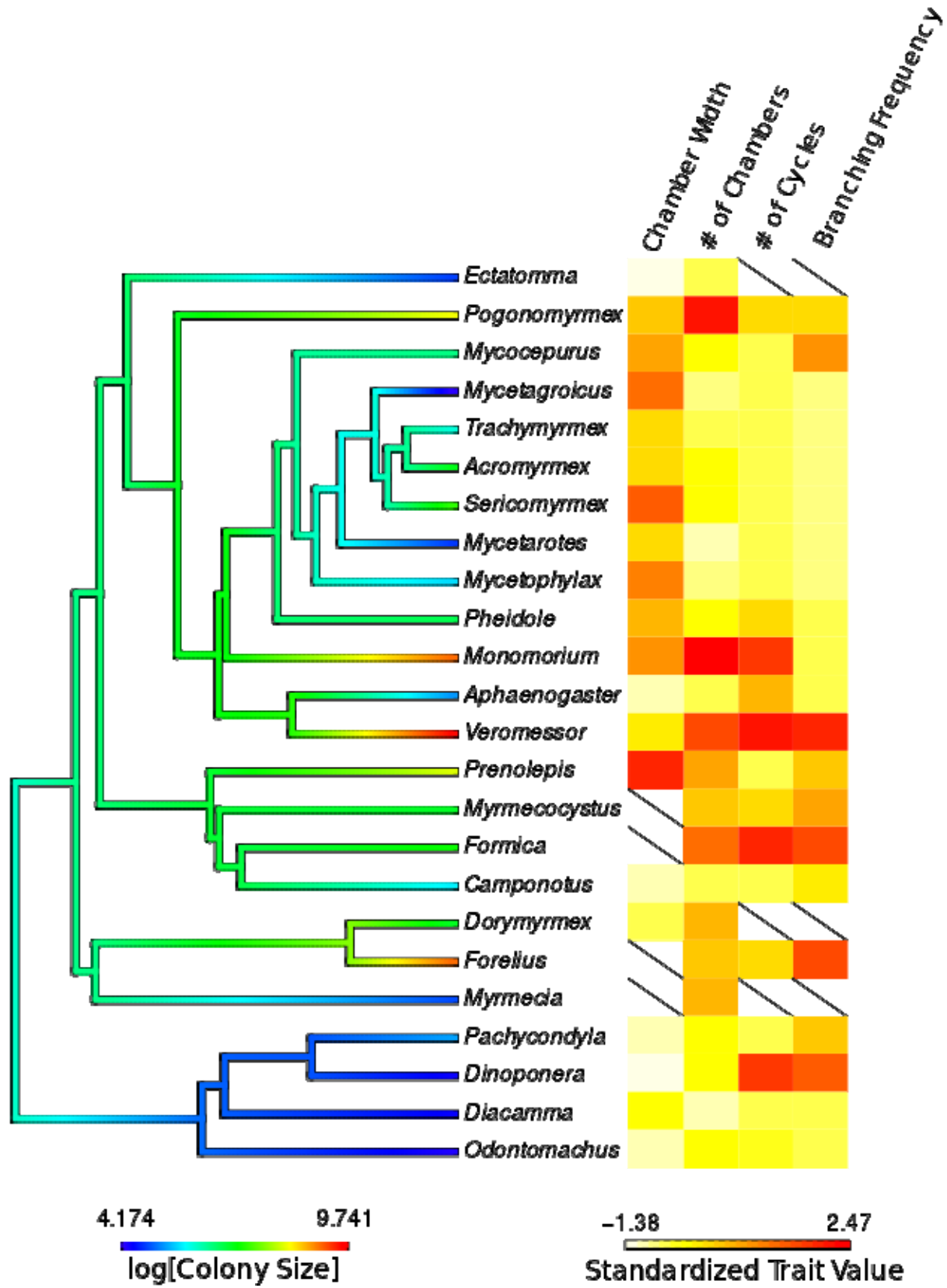
398 chain (red), MST (yellow), and triangulated (blue) reference networks, with 95% confidence
399 intervals from 1000 simulation runs shown as shading around the lines; in (E) Blue line is the
400 number of cycles in triangulated networks as a function of number of nodes. Orange line depicts
401 the number of cycles for both MSTs and chain networks, which contain zero cycles at all
402 network sizes; (C, F) Relationship between log colony size (average number of ants in a colony)
403 and scaled mean distance (C) and scaled number of cycles (F). Mean distance (C) is scaled to
404 chain (red), MST (yellow), and triangulated (blue) networks. Cycles (F) are only scaled to
405 triangulated networks (blue). Each point represents a mean of scaled values from the nests of
406 each species. Grey dashed line at $y=1$ represents values at which observed nests contain the same
407 value as expected from the reference model. Values below the dashed line indicate networks that
408 have lower values than expected by the reference model. Values above the dashed line indicate
409 networks which have values greater than expected according to the reference model. Solid
410 colored lines indicate statistically significant regression fits for PGLS ($\lambda=1$).

411

412 **Phylogenetic Signal**

413 We detected a significant phylogenetic signal for nest connectivity traits (cycles and mean
414 distance) but not for colony size or nest subdivision traits (chamber number and size). Most
415 traits, except colony size and number of cycles, tended to fit a Brownian Motion model of
416 evolution, however the differences in fit between the alternative models (no signal and estimated
417 Pagel's lambda) were not significant (<4 AIC units). We did not detect a significant phylogenetic
418 signal for colony size (Table S14; $p = 0.348$, $\lambda = 0.440$, $AIC_{\lambda} = 99.03$) in any species subsets
419 (0/19). Colony size had a slightly better fit to the no signal model ($AIC_0 = 97.29$) relative to the
420 Brownian Motion model ($AIC_{BM} = 99.35$) in all species subsets (19/19 subsets). We did not

421 detect a significant phylogenetic signal for chamber number (Table S15; $p = 0.091$, $\lambda = 1.07$,
422 $AIC_{\lambda} = 182.72$) in all species subsets (19/19). Chamber number had a slightly better fit to the
423 Brownian Motion model ($AIC_{BM} = 180.09$) relative to the no signal model ($AIC_0 = 182.60$) in all
424 species subsets (19/19 subsets). We did not detect a significant phylogenetic signal for
425 standardized chamber size (Table S16; $p = 0.084$, $\lambda = 0.71$, $AIC_{\lambda} = 88.79$) in the majority of
426 species subsets (12/15). Chamber size had a slightly better fit to the Brownian Motion model
427 ($AIC_{BM} = 86.83$) relative to the no signal model ($AIC_0 = 89.02$) in most species subsets (13/15).
428 Nest mean distance normalized to a chain network had a significant phylogenetic signal (Table
429 S17; $p = 0.001$, $\lambda = 1.12$, $AIC_{\lambda} = -2.22$) and had a slightly better fit to the Brownian Motion
430 model ($AIC_{BM} = -4.97$) relative to the no signal model ($AIC_0 = -2.19$) in all species subsets
431 (16/16). The number of cycles had a significant phylogenetic signal (Table S18; $p = 0.005$, $\lambda =$
432 1.12 , $AIC_{\lambda} = 74.97$), however we could not distinguish between the fit with the Brownian Motion
433 model ($AIC_{BM} = 72.23$) and the no signal model ($AIC_0 = 72.39$) in most species subsets (13/16).



435 **Figure 4:** Nest traits and colony size mapped to a genus-level phylogeny of the ants for all
436 genera on which we had data. The log of colony size is mapped onto the branches of the
437 phylogeny, with cooler colors indicating smaller colony sizes and warmer colors larger colony
438 sizes. Values for the four nest traits we analyzed are depicted as a heatmap, with color indicating
439 trait value standardized as a z-score. Branching frequency is a measure of how much nests
440 branch relative to their size; such that values are nest Mean Distance standardized to Chain
441 networks with values multiplied by -1 to ease interpretation, so that larger values indicate greater
442 branching. Data are displayed for species subset one, and slashes indicate missing data.

443

444 **DISCUSSION**

445 Our results support the adaptive hypothesis that nest architecture mitigates the challenges of
446 larger colony sizes, i.e., more individuals in a colony. Our comparative analysis found that nests
447 of species with larger colonies are more subdivided and reduce travel distances through
448 increased branching. Overall, we did not find a strong effect of phylogeny, but our findings lay
449 out a foundation for future experimental work on the evolution of mechanisms that allow ant
450 colonies to optimize the structures of their nests.

451 As predicted by the subdivision hypothesis, the number of chambers was greater in
452 species with larger colonies and chamber size was not related to colony size (Figure 2). Thus, ant
453 nests have evolved from the basal single-chambered form to accommodate larger colonies by
454 adding repeating modules of similar sized chambers rather than increasing chamber (module)
455 size. These results suggest that nests of species with large colonies are divided into more sub-
456 units than nests of species with small colonies. This pattern of increased spatial subdivision in
457 ant nests is shared across a variety of living systems, particularly those that undergo an

458 evolutionary increase in body size. For instance, the mammalian brain subdivides into more
459 cortical units in lineages with larger brain sizes (Kaas 2000, 2017). At smaller biological scales,
460 similar patterns of increased spatial subdivision form the basis for the evolution of multi-
461 cellularity that made larger body sizes possible (Bonner 1998). Even man-made networks, like
462 cities become more subdivided as they increase in size (Anas et al. 1998). Given our
463 correlational findings, it remains unclear if subdivision in nests serves a similar function as it
464 does in these other systems. Further data are needed on the distribution of individuals throughout
465 nest chambers to determine if ants utilize chambers in a way that is consistent with subdivision
466 (e.g., Tschinkel 1999; Murdock and Tschinkel 2015). Nest subdivision may facilitate division of
467 labor through spatial segregation of tasks (Tofts and Franks 1992; Richardson et al. 2011;
468 Mersch et al. 2013), and slow down the spread of disease (Pie et al. 2004; Stroeymeyt et al.
469 2014, 2018). However, the only direct test of the benefit of nest subdivision showed that
470 subdivision does not increase brood rearing efficiency (Cassill et al. 2002; Tschinkel 2018).
471 Limits on chamber size may also be imposed by physiological processes such as gas exchange or
472 temperature regulation (Jones and Oldroyd 2006), or by structural constraints, because large
473 chambers may collapse. Thus, the causes and consequences of nest subdivision remain to be
474 tested.

475 The connectivity of nests changed according to colony size, but only one of our
476 hypotheses about nest connectivity was supported. The nests of species with larger colonies had
477 reduced travel distances due to their branching structure, consistent with the branching
478 hypothesis. In the nests of species with small colonies (i.e., up to ~1000 workers in nests
479 containing up to ~10 nodes), mean distances tended to match the values expected for chain
480 networks, and indeed the nests of many small colony species were chains. In the nests of species

481 with large colonies, mean travel distances were shorter than would be expected for chain
482 networks but greater than expected for MSTs, and far greater than expected for triangulated
483 networks (Figure 3B, C). These findings indicate that the nests of species with large colonies
484 have increased branching (i.e., are more tree-like) relative to the nests of species with smaller
485 colonies. These findings show that nest branching evolves in association with colony size, and
486 that increased branching is not necessarily a more “derived” trait or explained by phylogenetic
487 relatedness alone. For example, the nests of some early branching species (e.g. *Dinoponera*)
488 have relatively more branching than the nests of some later branching species (e.g. leaf-cutters,
489 *Mycetagnocious*; Figures 3C & 4). By increasing the frequency of branching, large colonies may
490 curb rising travel distances as their nests increase in size (i.e., contain more chambers). Networks
491 are subject to an inherent tradeoff, in which they cannot simultaneously minimize travel
492 distances (i.e., be highly interconnected) and minimize their number of edges (i.e., minimize
493 infrastructure costs). Increased branching is a common way for biological transport networks to
494 balance this tradeoff (e.g., ant foraging trails: Buhl et al. 2009; slime molds: Tero et al. 2010;
495 neural networks: Raj and Chen 2011; plant growth forms: Conn et al. 2017), especially as body
496 size, or population size, increases (Ringo 1991; Banavar et al. 1999; Ercsey-Ravasz et al. 2013;
497 Cabanes et al. 2015). While these previous studies have identified branching as a way to balance
498 the connectivity-infrastructure cost tradeoff in single species (i.e., over development or between
499 individuals), ours is the first study to show this pattern across lineages in an evolutionary
500 context. Branching is thus a general low-cost way for biological transport networks, including
501 ant nests, to maintain connectivity as they increase in size.

502 The short-cuts hypothesis, that nests from species with larger colonies will have more
503 cross-linking paths or cycles, was not supported. Although the number of cycles in a nest

504 increased with colony size, this increase was not statistically significant (Figure 3D). Cycles
505 were added at a slower rate than expected for a triangulated network (Figure 3E), which is not
506 surprising given that triangulations represent the maximum rate at which new cycles could be
507 added with increasing network size (for 2D networks). This finding lends further support to the
508 conclusion that the increase in nest connectivity with colony size is due to increased branching
509 and not the addition of short-cuts. While we did not find support for this adaptive function of
510 cycles, our results lend some support to the hypothesis that variation in cycles is due to
511 phylogenetic processes, like drift, evolutionary history, etc., as cycle number had a significant
512 phylogenetic signal. However, we caution that our analysis conservatively excludes nests that
513 might have the most cycles because we could not accurately infer the topology of the most
514 convoluted nests. Our inability to detect a significant increase in the number of cycles with
515 colony size could be due to this bias in our dataset. More refined imaging techniques could
516 provide a solution to this issue and expand future work on nest architecture in general.

517 The scarcity of cycles in the nests we measured suggest that the benefits of having
518 alternative access routes, which help maintain movement (Corson 2010; Katifori et al. 2010;
519 Blonder et al. 2018, Jyothi et al. 2016) and facilitate forager recruitment (Pinter-Wollman
520 2015a), might not outweigh the potential costs of additional tunnels. For instance, there are costs
521 to building and maintaining tunnels (da Silva Camargo et al. 2013; Monaenkova et al. 2015) and
522 there may be negative consequences of many cross-linking paths, including structural instability
523 or heightened exposure to risks, like pathogens (Scovell 1983; Viana et al. 2013; Stroeymeyt et
524 al. 2014, 2018), parasites (Scovell 1983), or predators (Jeanne 1975; LaPolla et al. 2002). Nests
525 are multi-purpose structures that have other functions, like thermoregulation, humidity control or
526 ventilation (Jones and Oldroyd 2006; King et al. 2015), which might suffer when nests have

527 many cycles. In systems where the costs of cross-linking paths are low, like the plasmodia of
528 slime molds, the mycelia of fungus, or the pheromone trails of ants, cycles are ephemeral and
529 created for exploration, yet they are pruned once resources are discovered (Bebber et al. 2007;
530 Buhl et al. 2009; Tero et al. 2010; Latty et al. 2011). Conversely, when the benefits to movement
531 are very high, cycles become more common as size increases, but this only appears to be true in
532 some man-made networks, like urban roads or computer routing networks (Levinson 2012;
533 Jyothi et al. 2016). Future investigation into the building costs of tunnels and the functional
534 consequences of interconnected nest structures will help reveal why ant nests do not contain
535 more short-cuts, or cycles.

536 While we found support for our adaptive hypotheses, we also found that phylogenetic
537 relatedness explains some of the variation in ant nest structural diversity. Nest traits relating to
538 connectivity (number of cycles and mean distance normalized to chain networks), had significant
539 phylogenetic signals, whereas traits relating to subdivision (chamber number and size) did not.
540 We caution, however, that we did not detect a strong effect of phylogeny overall. The results of
541 our regression analyses did not differ when we assumed a strong phylogenetic signal ($\lambda=1$) or
542 none at all ($\lambda=0$). Furthermore, the differences in fit between alternative models of evolution (no
543 signal, Brownian Motion, and estimated Pagel's lambda) were rarely large enough to robustly
544 distinguish between models, which may be due to lack of statistical power. Notably, we did not
545 detect a significant phylogenetic signal for colony size, even though prior studies of ant colony
546 size suggest that phylogenetic relatedness is a factor in colony size evolution (Boulay et al. 2014;
547 Ferguson-gow et al. 2014; Burchill and Moreau 2016). While we cannot reach robust
548 conclusions about the extent of phylogenetic effect on the traits we measured, the co-
549 evolutionary patterns we analyze here account for potential phylogenetic non-independence.

550 As with any meta-analysis, our dataset is constrained by the available literature. Many of
551 the nests we found in the literature were built in sandy soils, and so our analysis has an over-
552 representation of species adapted to the climate and ecology of sandy soils (Table S1). Fungus
553 growers are highly-represented in our analysis (represented by the crown spanning from
554 *Mycocephurus* to *Mycetophylax* in Figure 4), and constraints imposed by requirements to maintain
555 healthy fungus gardens could have influenced our findings. Additionally, because we do not
556 have data on the size or age of the colonies that built the majority of nests in our meta-analysis,
557 we might have included immature colonies that have not reached their full size. Further work
558 could investigate whether nest connectivity changes throughout development within a species in
559 natural environments (e.g., Tschinkel 1987, 2004, 2005, 2011) or whether there are species-
560 specific connectivity patterns that can be found at different ages.

561 In conclusion, we show that ant nests exhibit modular structures that minimize travel
562 distancesf in species with larger colonies. The similarity in how ant nests and the bodies of
563 unitary organisms respond to increasing size suggest that their shared architectural features,
564 branching transportation networks and compartmentalization, provide common solutions to the
565 challenges created by large size across the tree of life.

566

567 **REFERENCES**

568 Aguilar, J., D. Monaenkova, V. Linevich, W. Savoie, B. Dutta, H.-S. Kuan, M. D. Betterton, M.

569 A. D. Goodisman, and D. I. Goldman. 2018. Collective clog control: Optimizing traffic

570 flow in confined biological and robophysical excavation. *Science* (80-.). 361:672–677.

571 Anas, A., R. Arnott, and K. A. Small. 1998. Urban spatial structure. *Am. Econ. Assoc.* 36:1426–

- 572 1464.
- 573 Banavar, J. R., A. Maritan, and A. Rinaldo. 1999. Size and form in efficient transportation
574 networks. *Nature* 399:130–132.
- 575 Bebbler, D. P., J. Hynes, P. R. Darrah, L. Boddy, and M. D. Fricker. 2007. Biological solutions to
576 transport network design. *Proc. R. Soc. B Biol. Sci.* 274:2307–2315.
- 577 Blanchard, B. D., and C. S. Moreau. 2017. Defensive traits exhibit an evolutionary trade-off and
578 drive diversification in ants. *Evolution (N. Y.)* 71:315–328.
- 579 Blonder, B., N. Salinas, L. P. Bentley, A. Shenkin, P. O. Chambi Porroa, Y. Valdez Tejeira, T. E.
580 Boza Espinoza, G. R. Goldsmith, L. Enrico, R. Martin, G. P. Asner, S. Díaz, B. J. Enquist,
581 and Y. Malhi. 2018. Structural and defensive roles of angiosperm leaf venation network
582 reticulation across an Andes–Amazon elevation gradient. *J. Ecol.* 106:1683–1699.
- 583 Bonner, J. T. 2004. Perspective: The Size-Complexity Rule. *Evolution (N. Y.)* 58:1883–1890.
- 584 Bonner, J. T. 1988. *The Evolution of Complexity by Means of Natural Selection*. Princeton
585 University Press, Princeton, NJ.
- 586 Bonner, J. T. 1998. The origins of multicellularity. *Integr. Biol.* 1:27–36.
- 587 Boulay, R., X. Arnan, X. Cerdá, and J. Retana. 2014. The ecological benefits of larger colony
588 size may promote polygyny in ants. *J. Evol. Biol.* 27:2856–2863.
- 589 Bourke, A. F., and N. R. Franks. 1995. *Social Evolution in Ants*. Princeton University Press,
590 Princeton, NJ.
- 591 Branstetter, M. G., B. N. Danforth, J. P. Pitts, B. C. Faircloth, P. S. Ward, M. L. Buffington, M.
592 W. Gates, R. R. Kula, and S. G. Brady. 2017. Phylogenomic Insights into the Evolution of

- 593 Stinging Wasps and the Origins of Ants and Bees. *Curr. Biol.* 27:1019–1025.
- 594 Brian, M. V. 1956. Group Form and Causes of Working Inefficiency in the Ant *Myrmica rubra*
595 L. *Physiol. Zool.* 29:173–194.
- 596 Brown, J. J., and J. F. A. Traniello. 1998. Regulation of Brood-Care Behavior in the Dimorphic
597 Castes of the Ant *Pheidole morrisi* (Hymenoptera: Formicidae): Effects of Caste Ratio,
598 Colony Size, and Colony Needs. *J. Insect Behav.* 11:209–219.
- 599 Brown, W. L. 1953. Revisionary Studies in the Ant Tribe Dacetini. *Am. Midl. Nat.* 50:1.
- 600 Buhl, J., J. Gautrais, J.-L. Deneubourg, and G. Theraulaz. 2004a. Nest excavation in ants:
601 group size effects on the size and structure of tunneling networks. *Naturwissenschaften*
602 91:602–606.
- 603 Buhl, J., J. Gautrais, R. V. Sole, P. Kuntz, S. Valverde, J.-L. Deneubourg, and G. Theraulaz.
604 2004b. Efficiency and robustness of ant networks of galleries. *Eur. Phys. J. B* 129:123–129.
- 605 Buhl, J., K. Hicks, E. R. Miller, S. Persey, O. Alinvi, and D. J. T. Sumpter. 2009. Shape and
606 efficiency of wood ant foraging networks. *Behav. Ecol. Sociobiol.* 63:451–460.
- 607 Bullmore, E., and O. Sporns. 2012. The economy of brain network organization. *Nat. Rev.*
608 *Neurosci.* 13:336–349.
- 609 Burchill, A. T., and C. S. Moreau. 2016. Colony size evolution in ants: macroevolutionary
610 trends. *Insectes Soc.* 62:291–298.
- 611 Burd, M., N. Shiwakoti, M. Sarvi, and G. Rose. 2010. Nest architecture and traffic flow: large
612 potential effects from small structural features. *Ecol. Entomol.* 35:464–468.
- 613 Cabanes, G., E. Van Wilgenburg, M. Beekman, and T. Latty. 2015. Ants build transportation

- 614 networks that optimize cost and efficiency at the expense of robustness. *Behav. Ecol.*
615 26:223–231.
- 616 Calder, W. A. 1984. *Size, Function, and Life History*. Harvard University Press, Cambridge,
617 MA.
- 618 Cassill, D., W. R. Tschinkel, and S. B. Vinson. 2002. Nest complexity , group size and brood
619 rearing in the fire ant , *Solenopsis invicta*. *Insectes Soc.* 49:158–163.
- 620 Cheverud, J. M. 1982. Relationships among ontogenetic, static, and evolutionary allometry. *Am.*
621 *J. Phys. Anthropol.* 59:139–149.
- 622 Conn, A., U. V. Pedmale, J. Chory, and S. Navlakha. 2017. High-Resolution Laser Scanning
623 Reveals Plant Architectures that Reflect Universal Network Design Principles. *Cell Syst.*
624 5:53-62.e3. Elsevier Inc.
- 625 Corson, F. 2010. Fluctuations and Redundancy in Optimal Transport Networks. 048703:1–4.
- 626 Csárdi, G. 2020. Package ‘igraph’: Network Analysis and Visualization.
- 627 da Silva Camargo, R., J. F. S. Lopes, and L. C. Forti. 2013. Energetic cost of digging behavior in
628 workers of the leaf-cutting ant *Atta sexdens* (Fabricius). *Rev. Bras. Entomol.* 57:401–404.
- 629 Dial, K. P., E. Greene, and D. J. Irschick. 2008. Allometry of behavior. *Trends Ecol. Evol.*
630 23:394–401.
- 631 Dodds, P. S. 2010. Optimal form of branching supply and collection networks. *Phys. Rev. Lett.*
632 104:1–4.
- 633 Dornhaus, A., S. Powell, and S. Bengston. 2012. Group size and its effects on collective
634 organization. *Annu. Rev. Entomol.* 57:123–41.

- 635 Ercsey-Ravasz, M., N. T. Markov, C. Lamy, D. C. VanEssen, K. Knoblauch, Z. Toroczkai, and
636 H. Kennedy. 2013. A Predictive Network Model of Cerebral Cortical Connectivity Based
637 on a Distance Rule. *Neuron* 80:184–197.
- 638 Fairbairn, D. J. 1997. Allometry for sexual size dimorphism: Pattern and process in the
639 coevolution of body size in males and females. *Annu. Rev. Ecol. Syst.* 28:659–687.
- 640 Ferguson-gow, H., S. Sumner, A. F. G. Bourke, and K. E. Jones. 2014. Colony size predicts
641 division of labour in attine ants. *Proc. R. Soc. B Biol. Sci.* 281:20141411.
- 642 Frankino, W. A., B. J. Zwaan, D. L. Stern, and P. M. Brakefield. 2005. Natural selection and
643 developmental constraints in the evolution of allometries. *Science* (80-.). 307:718–720.
- 644 Freckleton, R. P. 2009. The seven deadly sins of comparative analysis. *J. Evol. Biol.* 22:1367–
645 1375.
- 646 Gillooly, J. F., C. Hou, and M. Kaspari. 2010. Eusocial insects as superorganisms: Insights from
647 metabolic theory. *Commun. Integr. Biol.* 3:360–362.
- 648 Gould, S. J. 1966. Allometry and size in ontogeny and phylogeny. *Biol. Rev. Camb. Philos. Soc.*
649 41:587–640.
- 650 Gould, S. J. 1971. Geometric Similarity in Allometric Growth: A Contribution to the Problem of
651 Scaling in the Evolution of Size. *Am. Nat.* 105:113–136.
- 652 Harmon, A. L., M. Pennell, C. Brock, J. Brown, W. Challenger, J. Eastman, R. Fitzjohn, R. Glor,
653 G. Hunt, L. Revell, G. Slater, J. Uyeda, and J. Weir. 2020. Package ‘geiger’: Analysis of
654 Evolutionary Diversification.
- 655 Hobson, E. A., M. J. Silk, N. H. Fefferman, D. B. Larremore, P. Rombach, S. Shai, and N.

- 656 Pinter-Wollman. 2021. A guide to choosing and implementing reference models for social
657 network analysis. *Biol. Rev.*, doi: 10.1111/brv.12775.
- 658 Holldobler, B., and E. O. Wilson. 2009. *The superorganism: the beauty, elegance, and*
659 *strangeness of insect societies* Title. W.W. Norton, New York.
- 660 Hölldobler, B., and E. O. Wilson. 1990. *The Ants*. Belknap Press, Cambridge, MA.
- 661 Huxley, J. 1932. *Problems of Relative Growth*. Methuen and Co., Ltd., London.
- 662 Jeanne, R. L. 1975. The Adaptiveness of Social Wasp Nest Architecture. *Q. Rev. Biol.* 50:267–
663 287.
- 664 Jones, J. C., and B. P. Oldroyd. 2006. Nest Thermoregulation in Social Insects. *Adv. In Insect*
665 *Phys.* 33:153–191.
- 666 Jyothi, S. A., A. Singla, P. B. Godfrey, and A. Kolla. 2016. Measuring and Understanding
667 Throughput of Network Topologies. *Int. Conf. High Perform. Comput. Networking, Storage*
668 *Anal. SC* 0:761–772.
- 669 Kaas, J. H. 2017. Evolution of the Brain, Cognition, and Emotion in Vertebrates. *Evol. Brain,*
670 *Cogn. Emot. Vertebr.* 59–80.
- 671 Kaas, J. H. 2000. Why is Brain Size so Important: Design Problems and Solutions as Neocortex
672 Gets Bigger or Smaller. *Brain Mind* 1:7–23.
- 673 Katifori, E., G. J. Szöllosi, and M. O. Magnasco. 2010. Damage and fluctuations induce loops in
674 optimal transport networks. *Phys. Rev. Lett.* 104:1–4.
- 675 Kempes, C. P., S. Dutkiewicz, and M. J. Follows. 2012. Growth, metabolic partitioning, and the
676 size of microorganisms. *Proc. Natl. Acad. Sci.* 109:495–500.

- 677 King, H., S. A. Ocko, and L. Mahadevan. 2015. Termite mounds harness diurnal temperature
678 oscillations for ventilation. *Proc. Natl. Acad. Sci.* 112:11589–11593.
- 679 Kleiber, M. 1947. Body size and metabolic rate. *Physiol. Rev.* 27:511–541.
- 680 Kwapich, C. L., G. Valentini, and B. Hölldobler. 2018. The non-additive effects of body size on
681 nest architecture in a polymorphic ant. *Philos. Trans. R. Soc. B* 373:20170235.
- 682 LaPolla, J. S., U. G. Mueller, M. Seid, and S. P. Cover. 2002. Predation by the army ant
683 *Neivamyrmex rugulosus* on the fungus-growing ant *Trachymyrmex arizonensis*. *Insectes*
684 *Soc.* 49:251–256.
- 685 Latty, T., K. Ramsch, K. Ito, T. Nakagaki, D. J. T. Sumpter, M. Middendorf, and M. Beekman.
686 2011. Structure and formation of ant transportation networks. *J. R. Soc. Interface* 8:1298–
687 1306.
- 688 Levinson, D. 2012. Network structure and city size. *PLoS One* 7:e29721.
- 689 Lorenz, D. M., A. Jeng, and M. W. Deem. 2011. The emergence of modularity in biological
690 systems. *Phys Life Rev.* 8:129–160.
- 691 McKown, A. D., H. Cochard, and L. Sack. 2010. Decoding leaf hydraulics with a spatially
692 explicit model: Principles of venation architecture and implications for its evolution. *Am.*
693 *Nat.* 175:447–460.
- 694 McMahon, T. A. 1975. Using body size to understand the structural design of animals:
695 quadrupedal locomotion. *J. Appl. Physiol.* 39:619–627.
- 696 McMahon, T. A., and J. T. Bonner. 1983. *On Size and Life*. Scientific American Books, Inc,
697 New York.

- 698 Mersch, D. P., A. Crespi, and L. Keller. 2013. Tracking individuals shows spatial fidelity is a
699 key regulator of ant social organization. *Science* (80-.). 340:1090–3.
- 700 Monaenkova, D., N. Gravish, G. Rodriguez, R. Kutner, M. A. D. Goodisman, and D. I.
701 Goldman. 2015. Behavioral and mechanical determinants of collective subsurface nest
702 excavation. *J. Exp. Biol.* 218:1295–1305.
- 703 Murdock, T. C., and W. R. Tschinkel. 2015. The life history and seasonal cycle of the ant,
704 *Pheidole morrisi* Forel, as revealed by wax casting. *Insectes Soc.* 62:265–280.
- 705 Newman, M. E. J. 2018. *Networks: An Introduction*. 2nd Editio. Oxford University Press,
706 Oxford.
- 707 Niklas, K. J., and S. A. Newman (eds). 2016. *Multicellularity: Origins and Evolution*. MIT Press,
708 Cambridge, MA.
- 709 O'Donnell, S., and S. J. Bulova. 2007. Worker connectivity: A review of the design of worker
710 communication systems and their effects on task performance in insect societies. *Insectes*
711 *Soc.* 54:203–210.
- 712 Okie, J. G. 2013. General models for the spectra of surface area scaling strategies of cells and
713 organisms: Fractality, geometric dissimilitude, and internalization. *Am. Nat.* 181:421–439.
- 714 Orme, A. D., R. Freckleton, G. Thomas, T. Petzoldt, N. Isaac, W. Pearse, and M. D. Orme. 2018.
715 Package ‘caper’: Comparative Analyses of Phylogenetics and Evolution in R.
- 716 Pacala, S. W., D. M. Gordon, and H. C. J. Godfray. 1996. Effects of social group size on
717 information transfer and task allocation. *Evol. Ecol.* 10:127–165.
- 718 Pagel, M. 1999. Inferring historical patterns of biological evolution. *Nature* 401:877–884.

- 719 Paradis, E., S. Blomberg, B. Bolker, J. Brown, J. Claude, H. S. Cuong, R. Desper, G. Didier, B.
720 Durand, J. Dutheil, R. Ewing, O. Gascuel, T. Guillaume, C. Heibl, A. Ives, B. Jones, F.
721 Krah, D. Lawson, V. Lefort, P. Legendre, J. Lemon, E. Marcon, R. McCloskey, J.
722 Nylander, R. Opgen-Rhein, A.-A. Popescu, M. Royer-Carenzi, K. Schliep, K. Strimmer,
723 and D. de Vienne. 2018. Package ‘ape’: Analyses of Phylogenetics and Evolution.
- 724 Perna, A., C. Jost, E. Couturier, S. Valverde, S. Douady, and G. Theraulaz. 2008. The structure
725 of gallery networks in the nests of termite *Cubitermes* spp. revealed by X-ray tomography.
726 *Naturwissenschaften* 95:877–884.
- 727 Perna, A., and T. Latty. 2014. Animal transportation networks. *J. R. Soc. Interface* 11.
- 728 Peters, R. H. 1983. *The Ecological Implications of Body Size*. Cambridge University Press.
- 729 Pie, M. R., R. B. Rosengaus, and J. F. A. Traniello. 2004. Nest architecture , activity pattern ,
730 worker density and the dynamics of disease transmission in social insects. *J. Theor. Biol.*
731 226:45–51.
- 732 Pinter-Wollman, N. 2015a. Nest architecture shapes the collective behaviour of harvester ants.
733 *Biol. Lett.* 11:20150695-.
- 734 Pinter-Wollman, N. 2015b. Persistent variation in spatial behavior affects the structure and
735 function of interaction networks. *Curr. Zool.* 61:98–106.
- 736 Pinter-Wollman, N., A. Bala, A. Merrell, J. Queirolo, M. C. Stumpe, S. Holmes, and D. M.
737 Gordon. 2013. Harvester ants use interactions to regulate forager activation and availability.
738 *Anim. Behav.* 86:197–207. Elsevier Ltd.
- 739 Pinter-wollman, N., A. Penn, G. Theraulaz, and S. M. Fiore. 2018. Interdisciplinary approaches

740 for uncovering the impacts of architecture on collective behaviour. *Philos. Trans. R. Soc. B*
741 373:20170232.

742 R Core Team. 2019. R: A language environment for statistical computing.

743 Raj, A., and Y. H. Chen. 2011. The wiring economy principle: Connectivity determines anatomy
744 in the human brain. *PLoS One* 6.

745 Revell, M. L. J. 2017. Package ‘phytools’: Phylogenetic Tools for Comparative Biology (and
746 Other Things).

747 Richardson, T. O., K. Christensen, N. R. Franks, H. J. Jensen, and A. B. Sendova-franks. 2011.
748 *Ants in a Labyrinth*: A Statistical Mechanics Approach to the Division of Labour. *PLoS*
749 *One* 6:e18416.

750 Ringo, J. L. 1991. Neuronal interconnection as a function of brain size. *Brain. Behav. Evol.*
751 38:1–6.

752 Rueden, C. T., J. Schindelin, M. C. Hiner, B. E. DeZonia, A. E. Walter, E. T. Arena, and K. W.
753 Eliceiri. 2017. ImageJ2: ImageJ for the next generation of scientific image data. *BMC*
754 *Bioinformatics* 18:1–26.

755 Ruppert, E. E., R. S. Fox, and R. D. Barnes. 2004. *Invertebrate Zoology: A Functional*
756 *Evolutionary Approach*. Thomson-Brooks/Cole.

757 Schmidt-Nielson, K. 1984. *Scaling: Why is Animal Size So Important?* Cambridge University
758 Press, New York.

759 Scovell, E. 1983. Dominance and reproductive success among slave-making worker ants. *Nature*
760 304:724–725.

- 761 Seeley, T. D. 1989. The honey bee colony as a superorganism. *Am. Sci.* 77:546–553.
- 762 Stroeymeyt, N., B. Casillas-Pérez, and S. Cremer. 2014. Organisational immunity in social
763 insects. *Curr. Opin. Insect Sci.* 5:1–15.
- 764 Stroeymeyt, N., A. V Grasse, A. Crespi, D. P. Mersch, S. Cremer, and L. Keller. 2018. Social
765 network plasticity decreases disease transmission in a eusocial insect. *Science* (80-.).
766 362:941–945.
- 767 Tekin, E., D. Hunt, M. G. Newberry, and V. M. Savage. 2016. Do Vascular Networks Branch
768 Optimally or Randomly across Spatial Scales? *PLoS Comput. Biol.* 12:e1005223.
- 769 Tero, A., S. Takagi, T. Saigusa, K. Ito, D. P. Bebbler, M. D. Fricker, K. Yumiki, R. Kobayashi,
770 and T. Nakagaki. 2010. Rules for biologically inspired adaptive network design. *Science*
771 (80-.). 327:439–442.
- 772 Thompson, D. W. 1917. *On Growth and Form*. Cambridge University Press, Cambridge, MA.
- 773 Tofts, C., and N. R. Franks. 1992. Doing the right thing: Ants, honeybees and naked mole-rats.
774 *Trends Ecol. Evol.* 7:346–349.
- 775 Tschinkel, W. R. 2010. Methods for casting subterranean ant nests. *J. Insect Sci.* 88:1–17.
- 776 Tschinkel, W. R. 1987. Seasonal life history and nest architecture of a winter-active ant,
777 *Prenolepis imparis*. *Insectes Soc.* 34:143–164.
- 778 Tschinkel, W. R. 1999. Sociometry and sociogenesis of colonies of the harvester ant,
779 *Pogonomyrmex badius*: distribution of workers, brood and seeds within the nest in relation
780 to colony size and season. *Ecol. Entomol.* 24:222–237.
- 781 Tschinkel, W. R. 2003. Subterranean ant nests: trace fossils past and future? *Palaeogeogr.*

- 782 Palaeoclimatol. Palaeoecol. 192:321–333.
- 783 Tschinkel, W. R. 2018. Testing the effect of a nest architectural feature in the fire ant *Solenopsis*
784 *invicta* (Hymenoptera: Formicidae). *Myrmecological News* 27:1–5.
- 785 Tschinkel, W. R. 2015. The architecture of subterranean ant nests: beauty and mystery underfoot.
786 *J. Bioeconomics* 17:271–291.
- 787 Tschinkel, W. R. 2005. The nest architecture of the ant *Camponotus socius*. *J. Insect Sci.* 5:1–
788 18.
- 789 Tschinkel, W. R. 2004. The nest architecture of the Florida harvester ant, *Pogonomyrmex*
790 *badius*. *J. Insect Sci.* 21:1–19.
- 791 Tschinkel, W. R. 2011. The nest architecture of three species of north Florida *Aphaenogaster*
792 ants. *J. insect Sci.* 11:1–30.
- 793 Turner, R. 2020. Package ‘deldir’: Delaunay Triangulation and Dirichlet (Voronoi) Tessellation
794 Author.
- 795 Vaes, O., A. Perna, and C. Detrain. 2020. The effect of nest topology on spatial organization and
796 recruitment in the red ant *Myrmica rubra*. *Sci. Nat.* 107. *The Science of Nature*.
- 797 Viana, M. P., V. Fourcassié, A. Perna, F. Costa, and C. Jost. 2013. Accessibility in networks□:
798 A useful measure for understanding social insect nest architecture. *Chaos, Solitons and*
799 *Fractals* 46:38–45. Elsevier Ltd.
- 800 West, G. B., J. H. Brown, and B. J. Enquist. 1997. A General Model for the Origin of Allometric
801 Scaling Laws in Biology. *Science* (80-.). 276:122–126.
- 802 Wheeler, W. M. 1910. *Ants: Their structure, Development and Behavior*. Columbia University

803 Press, New York.

804