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1 **Manipulation of worker size diversity does not affect colony fitness under natural**
2 **conditions in the ant *Temnothorax nylanderi***

3

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16

17 **Abstract**

18 In social insects, within-colony worker diversity has long been thought to improve colony
19 fitness. Recent studies start to question this assertion in species with moderate diversity.
20 However, demonstrating an absence of effect is difficult as this absence could be due to the
21 measurement of only few life history traits or to artificial conditions. In order to circumvent
22 these limitations, we experimentally decreased worker size diversity within colonies of the ant
23 *Temnothorax nylanderi*, with or without affecting mean worker size and we reintroduced
24 them in the field for four months (spring and early summer). We then thoroughly measured
25 their fitness based on survival, growth and reproductive success. Our results show that our

26 manipulation did not affect colony fitness. In addition, colonies did not restore diversity to its
27 initial level, further suggesting that worker size diversity is not a key parameter to them. We
28 found the classically observed positive relationship between colony size, colony growth and
29 reproductive success. Overall, our results confirm that worker size diversity within colony is
30 not necessarily adaptive in species where it is moderate. We discuss the alternative
31 mechanisms that could explain the evolutionary persistence of moderate worker size diversity.

32

33 **Significance statement**

34 Organisms that live in groups can greatly benefit from the emergence of novel group-level
35 traits. For instance, social insects show significant variability in worker size within colonies.
36 This size diversity increases the division of labour among workers and improves colony
37 fitness. However, in species where size diversity is moderate, this relationship may not always
38 be verified. Here, we manipulated both worker size diversity and mean worker size within
39 colonies of the ant *Temnothorax nylanderi*, we reintroduced them in the field, and we
40 extensively measured colony fitness after four months. We found no impact on colony
41 survival, growth and reproduction. We discuss how social life and its associated cooperation
42 and conflicts could cause variation in worker size, without any positive effect on colony
43 fitness.

44

45 **Key-words:** colony size, social insects, mean body size, survival, growth, reproductive
46 success

47

48 **Introduction**

49

50 Animal societies can exhibit genetic, behavioral, physiological and morphological diversity
51 among individuals of the same group and this is thought to provide benefits to the group
52 (Sendova-Franks and Franks 1999; Robinson 2009). One of the most obvious and
53 ecologically important traits in social insects is size diversity of individuals within colonies
54 (bees: Brand & Chapuisat 2012; Linksvayer et al. 2011 - wasps: Kovacs et al. 2010 - ants:
55 Molet et al. 2012). Worker size diversity improves division of labour and colony efficiency in
56 many species (e.g. bumble bee: Peat et al. 2005; stingless bee: Ramalho et al. 1998). Among
57 social insects, ants are the most morphologically diverse group and the one where the highest
58 level of morphological diversity within colonies can be observed (Wheeler 1991). In ant
59 species with high worker polymorphism (including discrete worker and soldier castes),
60 studies support a general positive relationship between worker size diversity and colony
61 performance (Davidson 1978; Wilson 1984; Yang et al. 2004; Evison et al. 2008; Powell
62 2009; Arnan et al. 2011 - but see: Rissing and Polloek 1984; Calabi and Traniello 1989).
63 However, most species have moderate worker size diversity (with an intra-colonial coefficient
64 of variation lower than 0.2 (Fjerdingstad and Crozier (2006))), and in this case the link
65 between worker size diversity and colony performance is less clear. (Beshers and Traniello
66 1994, 1996) suggest that the adaptiveness of size variation in moderately polymorphic ants is
67 unrelated to division of labor, and size could be driven by other pressures which increase
68 survival or colony growth. Some studies show a positive relationship (Porter and Tschinkel
69 1985a, 1986; Heinze et al. 2003; Billick and Carter 2007; Modlmeier et al. 2013), others do
70 not (Billick 2002; Modlmeier and Foitzik 2011; Westling et al. 2014; Yilmaz et al. 2019). In
71 ant species with moderate worker size diversity, within group morphological diversity could
72 therefore be non-adaptive at the colony level.

73 However, it is difficult to reliably rule out any adaptive value of worker size diversity
74 as the presence or the lack of positive effects could always result from the limited number of

75 traits investigated or the rearing setup under artificial laboratory conditions. Indeed, some
76 studies focus on a single trait, such as foraging (Billick and Carter 2007; Westling et al.
77 2014), brood production (Porter and Tschinkel 1985a, 1986; Billick 2002; Modlmeier and
78 Foitzik 2011), thermal tolerance (Heinze et al. 2003; Yilmaz et al. 2019) or resistance to
79 starvation (Modlmeier et al. 2013). Accordingly, trade-offs with other traits that could be
80 negatively affected by size diversity may have been overlooked, resulting in a biased
81 assessment of fitness. Moreover, some of these studies are correlative, so they do not prove a
82 causal link between size diversity and colony efficiency (e.g. Modlmeier and Foitzik 2011;
83 Westling et al. 2014). Finally, all the experimental studies were performed in the laboratory
84 where realistic environmental pressures are lacking (e.g. Porter and Tschinkel 1985a; Colin et
85 al. 2017). Indeed the distinct environmental conditions (photoperiod, temperature, food
86 availability, social environment) of laboratory and field experiments cause variation in the
87 behavior of both vertebrates and invertebrates (Campbell et al. 2009).

88 Size diversity and mean worker size increase with colony size (respectively: Ferguson-
89 Gow et al. 2014; Porter and Tschinkel 1985b). Only colonies with sufficient resources can
90 produce large workers and enhance worker size diversity. It is important to disentangle these
91 two traits in order to properly conclude about the adaptive value of size diversity and mean
92 worker size within colonies of social insects. Indeed, a recent study on bumblebee colonies
93 highlighted a positive correlation between colony performance and mean worker size, but no
94 link with worker size diversity (Herrmann et al. 2018).

95 In this study, we aimed at quantifying the impact of within-colony worker size
96 diversity and worker mean size on colony fitness using a long-lasting field-based experiment
97 with extensive measures of fitness (survival, growth and reproductive success). We focused
98 on an ant species lacking discrete worker subcastes and exhibiting moderate worker size
99 diversity, *Temnothorax nylanderii* (see Appendix A1). In *Temnothorax* species, worker size

100 does not determine worker specialization (Pinter-Wollman et al. 2012), task-allocation is
101 highly flexible (Robinson, Feinerman, & Franks 2009) and it relies on physiological
102 parameters (Robinson et al. 2012). Colin et al. (2017) manipulated worker size diversity
103 within *T. nylanderi* colonies and challenged colonies in the laboratory with various tasks (nest
104 emigration, foraging, hygienic behavior, cold shock, etc.). They did not find any differences
105 in performance between colonies with reduced size diversity and control colonies with
106 unmanipulated diversity. Moreover, neither mean size nor size diversity correlate with per-
107 capita productivity (Modlmeier and Foitzik 2011). This suggests that worker size diversity is
108 not adaptive in this species, although larger individuals survive longer to cold temperature
109 (Heinze et al. 2003) and starvation (Modlmeier et al. 2013). We tested whether this holds true
110 in the field by manipulating the size of workers in colonies in the laboratory. Importantly, we
111 manipulated both within colony worker size diversity (decrease) and mean worker size
112 (increase, decrease or no change) in order to disentangle their effects. We subsequently re-
113 implanted colonies in the field for four months to expose them to natural biotic and abiotic
114 selective pressures, and we subsequently measured colony fitness extensively. In
115 *Temnothorax* species, brood production is highly synchronized (Headley 1943; Kipyatkov
116 1993) so, in addition to colony survival, we were able to quantify two additional fitness
117 parameters, namely colony growth (based on the production of new workers) and
118 reproductive success (based on the production of sexuals). In addition, because workers
119 control larval development (Linksvayer et al. 2011), we expected colonies to compensate for
120 the size of the removed individuals and restore diversity by producing workers of the very
121 same size if size diversity is somewhat important for colony fitness.

122

123 **Material and methods**

124

125 *Ants*

126 Colonies of *Temnothorax nylanderi* nest in twigs or acorns and are thus easy to pick up from
127 the forest leaf litter. One hundred and forty-six colonies of *T. nylanderi* were collected in
128 February 2019 in the 'Bois de Vincennes' forest (Paris, France, 48° 50' 22.14" N, 2°26'
129 51.96" E). In the laboratory, each colony was installed in an artificial nest consisting of two
130 microscope slides separated by a 1-mm auto-adhesive plastic foam with three chambers. The
131 nest was placed in a plastic box (11.5 × 11.5 × 5.5 cm) providing a foraging area (see
132 Appendix A2). During their two-week stay in the laboratory, colonies were kept in a climatic
133 chamber at 10-12°C with a natural photoperiod mimicking the natural conditions at this
134 period. Water was provided *ad libitum* in a tube plugged with cotton. Colonies were fed once
135 with a freshly killed mealworm (*Tenebrio molitor*). Workers and brood were counted.

136

137 *Manipulation of worker size*

138 Out of the 146 colonies, 80 colonies were selected for the experiment based on the presence
139 of one queen and at least 70 workers. Four treatments were performed and, in all treatments,
140 50% of workers were removed from the 20 colonies. In treatment 25S25L, we decreased
141 worker diversity without changing mean worker size by removing the 25% smallest workers
142 and the 25% largest workers. In the two other treatments, we decreased worker diversity but
143 also either increased mean worker size by removing the 50% smallest workers (treatment
144 50S) or decreased mean worker size by removing the 50% largest workers (treatment 50L).
145 The last treatment was the control (treatment 50R) as we removed 50% of workers randomly.
146 The worker removal protocols were similar to Colin et al. (2017) and based on the apparent
147 global body size: the sorting of large and small workers was done by eye under a stereoscopic
148 microscope (Zeiss®, x50 magnification) whereas the removal of random workers was done
149 without a microscope to make sure that worker size could not be evaluated. This method was

150 proven to be reliable (Colin et al. 2017). Colonies were assigned to the four treatments based
151 on their number of workers in order to keep a similar distribution of colony sizes among the
152 four treatments Workers remaining in the colonies after manipulation constituted the initial
153 workforce of the colonies ($67.8 \text{ workers} \pm 22.5$). Colonies initially contained $91.6 \text{ larvae} \pm$
154 27.7 .

155

156 *Colony rearing in the field*

157 We manufactured artificial nests to make them match natural nests as closely as possible, so
158 that colonies could easily live in them after reintroduction in nature. For that purpose, we used
159 2.5x 2cm truncated cone corks. We drilled a 4, 5 or 6mm-wide chamber from the larger side
160 of the cone cork, and plugged this side with a glue gun to seal the chamber. Then, a 1mm-
161 wide entrance gallery was pierced from the smaller side of the cone cork using a pointed plier
162 in order to connect the chamber to the outside. In the laboratory, six corks (two of each size)
163 were placed inside each plastic box, and we induced the emigration of colonies into the corks
164 of their choice by removing the cover glass of the original nest. Then, the six corks from each
165 plastic box (one containing the colony) were reintroduced in the ‘réserve ornithologique du
166 Bois de Vincennes’ in a semi-buried (10cm deep) 40 x 35cm bucket with a pierced bottom
167 (for water draining) and containing local soil (see Appendix A3). The bucket lid was cut off
168 into a ring shape and the bottom side was covered with fluon®, a slippery coating, to prevent
169 ant escape while retaining a wide entrance. The six corks were place randomly. This allowed
170 colonies to relocate to the nest of their choice (size and location) inside the bucket whenever
171 they wanted to. Indeed, *T. nylanderi* colonies readily switch nest depending on environmental
172 conditions (Foitzik et al. 2003). Because the 80 buckets had been in place for a year, the soil
173 and litter in the buckets were very similar to those of the surrounding forest; many arthropods
174 and soil organisms could come in and out of the buckets. A preliminary food-supplementation

175 experiment showed that adding food into the buckets did not increase colony survival and
176 growth, so the 40cm-wide foraging area provided enough food for the colonies. Colonies
177 were left in the buckets from March 26th to July 16th, and subsequently collected and brought
178 back to the laboratory. Corks were collected in the early morning, when workers were
179 inactive because of cool temperature, in order to make sure that complete colonies were
180 collected. Cork nests were destroyed and colonies were forced to move to artificial
181 microscope-slide nests. Workers, sexual and worker pupae and larvae were counted. There
182 were no sexual adults yet. Brood production is highly synchronized during Summer (July-
183 August) in *Temnothorax* (Headley 1943; Kipyatkov 1993; Modlmeier and Foitzik 2011),
184 therefore the individuals that we recovered as pupae most likely reflected the total production
185 of the colonies. We allowed pupae to molt into adults in order to assess the sex of the brood.
186 To do so, colonies were kept in the laboratory at room temperature ($21^{\circ}\text{C} \pm 2^{\circ}\text{C}$) with a
187 natural photoperiod. Water was provided *ad libitum* and colonies were fed once a week with
188 half a freshly killed mealworm. Emerging adults were killed by freezing and then stored in
189 96° ethanol. The workers, males and gynes counted as ‘newly produced’ by colonies were
190 individuals that emerged in the laboratory after developing into pupae in the field. All adults
191 had emerged by August 16th and all remaining individuals were killed.

192 We considered that colonies had survived if they were recovered in July and if their
193 queen was still alive. This loose definition of survival is an over-estimate relative to the
194 proper life-history trait ‘colony survival’, as some colonies may actually have escaped from
195 the bucket (entirely or only a fragment containing the queen, although this is highly unlikely
196 because the bottom side of the lid was covered with a slippery coating – see Methods), and
197 some colonies could have recovered from orphanage by later adopting a new fertilized queen
198 (Foitzik and Heinze 2000). Queenless colonies were excluded from the analyses. Mortality
199 rate of adult workers was computed as $1 - (\text{number of workers retrieved on July 16th} / \text{initial})$

200 workforce). Indeed, it is very unlikely that any workers were produced during this period
201 (Headley 1943; Kipyatkov 1993; Modlmeier and Foitzik 2011). We also counted and
202 measured the workers and sexuals (gynes and males) produced out of the pupae brought back
203 to the laboratory. Traits related to colony growth were the mortality rate of adult workers, the
204 number of workers produced, the mean size of the workers produced and its coefficient of
205 variation (CV). Traits related to reproductive success were the number of produced sexuals
206 (gynes and males), the mean size of the sexuals, and the sexual investment (number of sexuals
207 / number of workers produced). All three main fitness components of colonies were thus
208 measured.

209

210 *Morphological measurements*

211 The workers and sexuals that emerged in the laboratory were measured. Heads were separated
212 from bodies. Heads were stuck on double-sided tape and photographed under a
213 Discovery.V12 Zeiss stereomicroscope connected to a computer via a Zeiss AxioCam ICc 5
214 video camera. Head width, a standard measurement of body size in ants (Tschinkel et al.
215 2003), was measured with ImageJ 1.8 (Abràmoff et al. 2004) and measurements were blind to
216 treatment.

217

218 *Statistical analysis*

219 We investigated the effect of treatments (predictor variable, four levels) on colony survival,
220 traits related to colony growth (mortality rate of adult workers, number of new workers
221 produced, size of the produced workers and CV of the size of produced workers) and
222 reproductive success (number of produced sexuals, size of the sexuals and its CV, sexual
223 investment). The mortality rate of adult workers and the number of produced individuals were
224 also used as predictor variables in some models (Tables 1 and 2). So, in addition to

225 treatments, we considered four other predictor variables: the initial workforce (number of
226 workers in a colony after the experimental removal of 50% of workers), the initial number of
227 larvae in March, the mortality rate of adult workers and the number of produced workers and
228 sexuals. All first-order interactions between treatment and these variables were also included.

229 All statistical analyses were carried out with R v3.6.1 (www.r-project.org). All plots
230 were generated using *ggplot2* (Wickham 2009). First, we tested the effect of predictor
231 variables on colony-level dependent variables (mortality rate of adult workers, number of
232 produced workers and males, sexual investment, and size and CV of the produced
233 individuals) using generalized linear models (GLMs). Counting data (number of produced
234 individuals and sexual investment) were analyzed using quasipoisson distribution. Mortality
235 rate of adult workers and CV were analyzed using Gaussian distribution. Finally, we tested
236 the effect of predictor variables on the head size of emerged individuals, an individual-level
237 dependent variable, using linear mixed models (LMMs, package *nlme*; Pinheiro et al 2016),
238 with colony as random factor. All models are summarized in Table 1 and 2.

239 For each analysis, the minimum adequate model was selected using a backward
240 stepwise approach where predictor variables were removed one by one from a full model
241 based on a log likelihood ratio test. We used log likelihood ratio tests, i.e. F-tests for Gaussian
242 distribution or Chi^2 tests for quasi-poisson distribution, to obtain the *P*-values for each
243 predictor variable by comparing the minimum adequate model with a model excluding or
244 including the variable of interest (according to whether the variable was present in or absent
245 from the minimal adequate model respectively). Normality of the residuals and homogeneity
246 of variances were checked visually following Pinheiro and Bates (2000); no transformation of
247 the data was necessary.

248

249 **Results**

250

251 *Effect of treatments on colony survival*

252

253 Only five of the 80 manipulated colonies could not be recovered during colony collection in
254 July (three colonies 50S, one colony 50L and one colony 25L25S). Six of the 75 recovered
255 colonies had lost their queen (three colonies 50R and three colonies 50L). These queenless
256 colonies were excluded from the statistical analysis. Our final dataset consisted of 69
257 queenright colonies.

258

259 *Effect of treatments on growth*

260

261 The mortality rate of adult workers depended on the initial number of larvae in
262 interaction with the treatment (treatment x initial larvae interaction, $F_{57:60} = 2.95$, $P = 0.04$).
263 The mortality rate of adult workers decreased with the initial number of larvae with a steeper
264 slope in treatment 50S (figure 2a). The mortality rate of adult workers did not differ among
265 the treatments ($F_{57:66} = 1.94$, $P = 0.063$; figure 1a). Predictors that had a marginal effect ($0.05 <$
266 P -value < 0.07) and could therefore slightly affect the dependent variable were retained in the
267 minimum adequate model.

268

269 A total of 4 341 workers emerged in the laboratory from 66 queenright colonies (3
270 colonies did not produce any worker). The number of produced workers was positively
271 correlated with the initial workforce ($F_{66:67} = 51.81$, $P < 0.001$; figure 2b) and negatively
272 correlated with the mortality rate of adult workers ($F_{66:67} = 4.44$, $P = 0.04$), without
273 significant interaction with the treatments (see Table 1). The number of produced workers did
274 not differ among the treatments ($F_{66:63} = 0.38$, $P = 0.8$; figure 1b). The coefficient of variation
of the size of produced workers did not differ among the treatments ($F_{63:60} = 0.39$, $P = 0.8$),

275 and it was not explained by any other predictor (see Table 1). The size of produced workers
276 increased with the number of produced workers (mixed effect model: $\chi^2_1 = 38.51$, $P < 0.001$;
277 figure 3a) and with the initial number of larvae ($\chi^2_1 = 3.79$, $P = 0.05$). The treatments had no
278 effect on the size of produced workers ($\chi^2_3 = 3.25$, $P = 0.3$; figure 1d). Detailed statistics are
279 presented in Table 1.

280

281 *Effect of treatments on reproductive success*

282

283 No gynes were produced except for three in one colony of treatment 50S. However, 57
284 queenright colonies produced 809 males. The number of males was positively correlated with
285 the initial workforce ($F_{67:68} = 20.11$, $P < 0.001$; figure 2c) but was not affected by the
286 treatment ($F_{67:64} = 1.22$, $P = 0.3$). The size of produced males was positively correlated with
287 the number of males ($\chi^2_1 = 4.03$, $P = 0.04$; figure 3b). The sexual investment (number of
288 sexuals / number of produced workers) and the CV of the size of produced males were not
289 explained by any predictor. Detailed statistics are presented in Table 2.

290

291 **Discussion**

292

293 In this study, we aimed at quantifying the impact of worker size and size diversity within
294 colony on colony fitness using a laboratory manipulation followed by a reintroduction in the
295 field. Because brood production is synchronized in *Temnothorax nylanderi* (Headley 1943;
296 Kipyatkov 1993), we were able to measure both colony growth and sexual investment in
297 addition to survival, and thus to extensively quantify fitness. In agreement with a previous
298 laboratory study (Colin et al. 2017), reducing worker size diversity did not affect colony
299 fitness. Colony survival, reproductive success (the number of males and the investment in

300 sexuals) and colony growth (the number of produced workers) did not differ among the four
301 treatments (removal of large, small, large and small, or random workers). Surprisingly,
302 treatments that reduced or increased mean worker size did not affect fitness either. We also
303 found that colonies did not compensate for the size of the removed workers by producing
304 workers of the same size, which further suggests that worker size diversity has no strong
305 effect on colony fitness.

306 The initial workforce of colonies had a major impact on colony fitness in our
307 experiment, which is not the case in other species of the *Temnothorax* genus (Dornhaus et al.
308 2008; Bengston and Dornhaus 2013). Indeed, we found a positive relationship between the
309 initial workforce and the number of produced workers and males. This confirms that in *T.*
310 *nylanderi*, large groups grow and reproduce better (Foitzik and Heinze 2000; Foitzik et al.
311 2003). Only one colony produced gynes. This might be a consequence of the experimental
312 removal of half of the workers from each colony, which resulted in a loss of workforce and a
313 redirection of resources towards workers and males, as already known in *T. nylanderi* (Foitzik
314 and Heinze 2000; Foitzik et al. 2003).

315 Our results are in line with Colin et al. (2017), who found no impact of reducing
316 worker size diversity on colony performance in the laboratory in the same species, and
317 Modlmeier & Foitzik (2011) who found no correlation between worker size diversity and per
318 capita productivity in the field in *T. longispinosus*. Modlmeier & Foitzik (2011) also found no
319 effect of the mean worker size on colony productivity. Our experimental manipulation of
320 mean worker size confirms that it does not affect colony fitness. Moreover, colonies do not
321 compensate for the size of the removed workers, reinforcing the idea that worker size
322 diversity is not of primary value to them. Billick (2002) found a similar pattern in *Formica*
323 *neorufibarbis*. He collected 45 workers from field colonies, and removed the 15 largest
324 workers (7% of colony size). He followed colony development for 35 days and found no

325 consequence on the number of pupae produced per worker. In addition, although a positive
326 relationship between initial workforce and both mean worker size and worker size diversity is
327 usually found in ants (Porter and Tschinkel 1985a; Ferguson-Gow et al. 2014), we did not
328 find such a correlation. Our results hence confirm that this trait is not of primary importance
329 for colonies. For the first time, our study proves the absence of effect of both mean worker
330 size and worker size diversity in the field, over a long period of time, and with an extensive
331 measure of colony fitness (three main life-history traits), making it a highly reliable result. We
332 discuss three non-mutually exclusive hypotheses that could explain why within-colony
333 worker size diversity still occurs.

334 First, worker size diversity could only bring advantages in the context of harsher
335 environmental conditions. In *T. nylanderii*, large workers resist better to starvation (Modlmeier
336 et al. 2013). In the closely related *Leptothorax acervorum*, larger individuals survive longer in
337 cold temperature (Heinze et al. 2003). Based on our results, such individual-level response
338 (individual survival) does not seem to translate to the group level (colony survival). Further
339 experiments are needed to fully explore the role of size diversity in the context of harsher
340 environmental conditions (e.g. during overwintering) or over a full life cycle.

341 Second, size diversity could result from relaxed selection. Group living can buffer
342 selective pressures on individual development because (1) phenotypes can diverge and still be
343 viable under the protection of the social environment (Molet et al. 2012) and (2) workers
344 themselves can control the environment of developing larvae and adjust their development if
345 needed (Smith et al. 2008; Segers et al. 2015). This could thus decrease the level of
346 canalization (the ability of development to remain unchanged despite environmental or
347 genetic variation; Debat and David 2001), and thereby increase worker size diversity. The
348 cost of maintaining developmental canalization (Van Buskirk and Steiner 2009) could be
349 outbalanced by the lack of benefits that it brings in a social context. Accordingly, size

350 diversity, in species where it is moderate, could be not adaptive *per se* but just economical to
351 avoid the costs of canalization (Hunt et al. 2011; Colin et al. 2017).

352 Third, larvae could exhibit selfish strategies (Amor et al. 2011, 2016). Larvae may
353 seek to receive more resources in order to become larger individuals, and increase their direct
354 fitness by laying male eggs (Stroeymeyt et al. 2007). Heinze & Oberstadt (1999) showed that
355 large workers of the ant *Leptothorax gredleri* (closely related to *T. nylanderi*) have higher
356 social ranks than small ones, so they are more likely to be the ones that lay male eggs. It
357 would be interesting to compare larval development in the presence or absence of workers to
358 shed light on this potential conflict. In *Ectatomma tuberculatum*, larval begging does not fully
359 reflect the nutritional status, suggesting that larvae do cheat to receive more food (Sauvaget
360 2017).

361 The ecological success of *T. nylanderi* (up to 10 colonies per m²: Heinze et al. 1996 -
362 with a wide distribution throughout Western European forests, woods and parks: Csoz et al.
363 2015; Khimoun et al. 2020), probably originates from unassessed traits other than mean
364 worker size or worker size diversity, such as behavioral diversity. Indeed, *Temnothorax* ants
365 demonstrate highly flexible and context-dependent task specialization, (Pinter-Wollman et al.
366 2012) and they can quickly respond to changes in the required tasks (Robinson et al. 2009).
367 Colonies with high within-colony behavioral diversity are more productive than colonies with
368 less variation (Modlmeier et al. 2012). This may apply more generally to social insects.
369 Indeed, in a ponerine ant lacking size diversity, age-associated division of labour is highly
370 flexible. Foragers can revert to nursing and egg laying (Bernadou et al. 2015). Moreover, the
371 functioning of honeybee societies is based on the coexistence of physiologically specialized
372 individuals that perform fixed functions such as nectar processing, and physiologically plastic
373 individuals that are also more flexible behaviorally (Johnson 2003). To summarize, the
374 ecological success of social insects with high size-diversity could come from their sharp

375 division of labour in relation with workers morphology (with the exception of *Veromessor*
376 *pergandei* and *Pheidole dentata*, respectively Rissing and Polloek 1984; Calabi and Traniello
377 1989), whereas the success of species with moderate size diversity could result from their
378 behavioral ability to quickly adapt to specific contexts.

379

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386

387 **Author's contributions**

388 RH designed the study, collected and reared ants, performed the experiment and statistical
389 analyses, and wrote the manuscript. CD designed the study, contributed to statistical analyses
390 and wrote the manuscript. MM designed the study, wrote the manuscript and supervised the
391 project. All authors read and approved the final manuscript.

392

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395 Institut de la Transition Environnementale de Sorbonne Université.

396

397 **Data availability**

398 The dataset analyzed during the current study is available from the corresponding author on
399 reasonable request.

400

401 **Compliance with ethical standards**

402

403 **Conflict of interest**

404 The authors declare that they have no conflict of interest.

405

406 **Ethical approval**

407 All applicable international, national and/or institutional guidelines for the care and use of
408 animals were followed.

409

410 **References**

411

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548

549 **Figure captions**

550

551 Figure 1: Boxplots comparing the effects of the four treatments (50R: 50% of randomly
552 removed workers; 25S25L: the 25% smallest and the 25% largest removed workers; 50L: the
553 50% largest removed; 50S: the 50% smallest removed) on four life history traits (a) mortality
554 rate of adult workers, (b) number of produced workers, (c) number of produced males and (d)
555 head size of produced workers (mm). Boxes show median, quartiles and extremes. Statistics
556 are presented in Table 1 for workers and Table 2 for males.

557

558 Figure 2: Correlation between the initial larvae and workforce with three different traits.
559 When the initial larvae increased, (a) the mortality rate of adult workers (50S: solid line type,
560 estimate = $-2.141e^{-3} \pm 1.14e^{-3}$; 50R: dotdash, estimate = $-1.072e^{-3} \pm 1.08e^{-3}$; 50L: dotted,
561 estimate = $-1.074e^{-3} \pm 1.16e^{-3}$; 25L25S: longdash, estimate = $-0.701e^{-3} \pm 1.03e^{-3}$). When the
562 initial workforce increased, (b) the number of produced workers increased similarly for all
563 treatments, and (c) the number of produced males increased similarly for all treatments. The
564 regression lines are drawn from the coefficients of the different models. Statistics are
565 presented in Table 1 for workers and Table 2 for males.

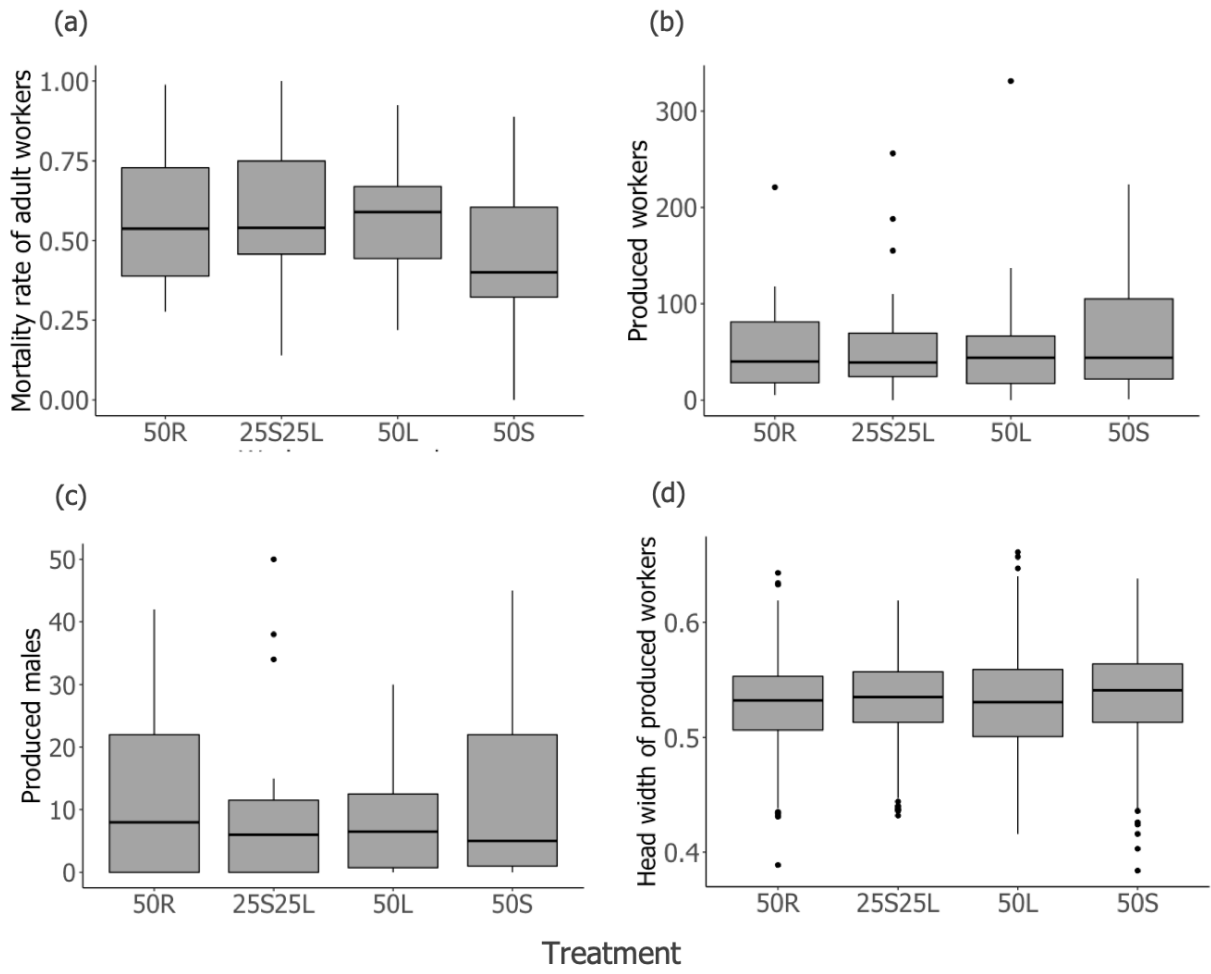
566

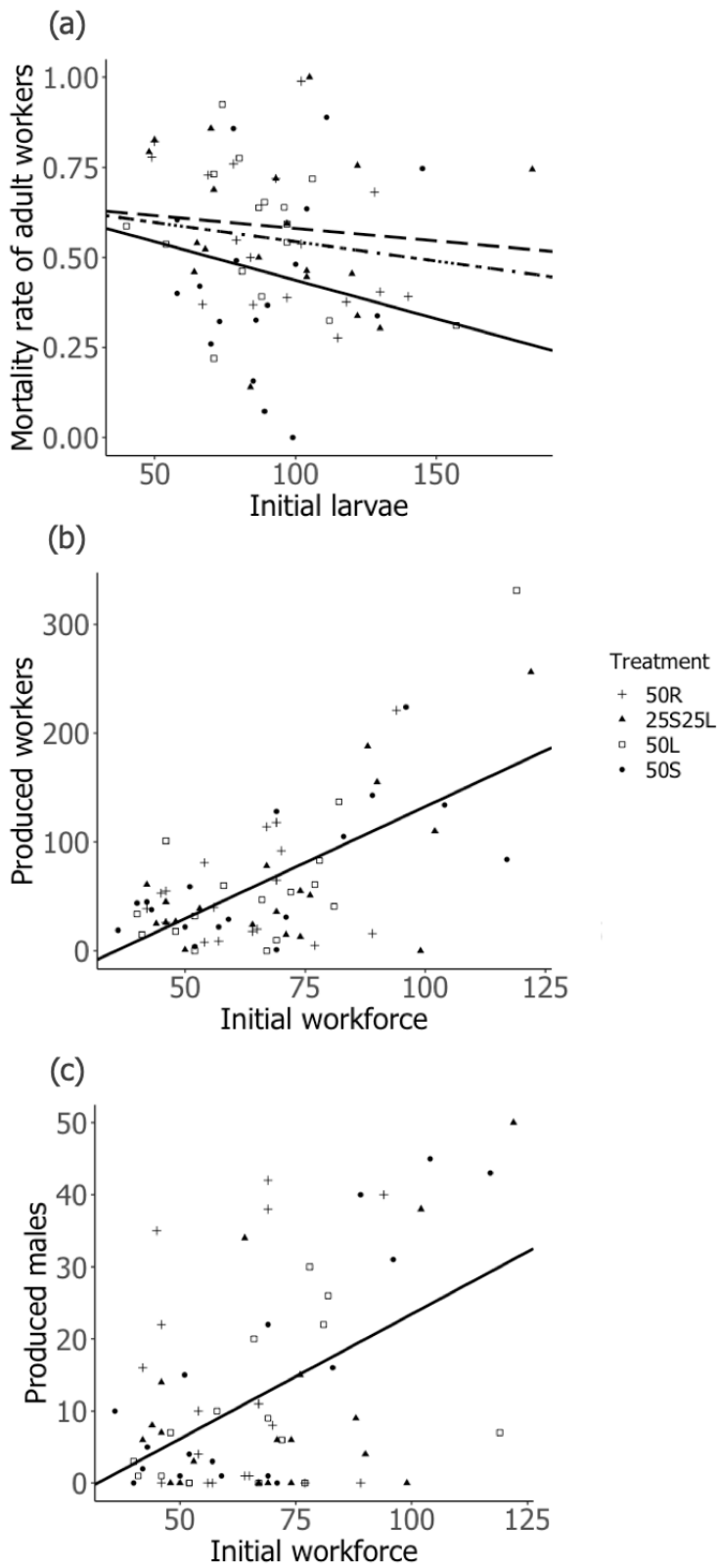
567 Figure 3: Correlation between the mean head size (mm) and the number of (a) produced
568 workers and (b) produced males. The regression lines are drawn from the coefficients of the
569 models and did not differ among treatments. Statistics are presented in Table 1 and Table 2.

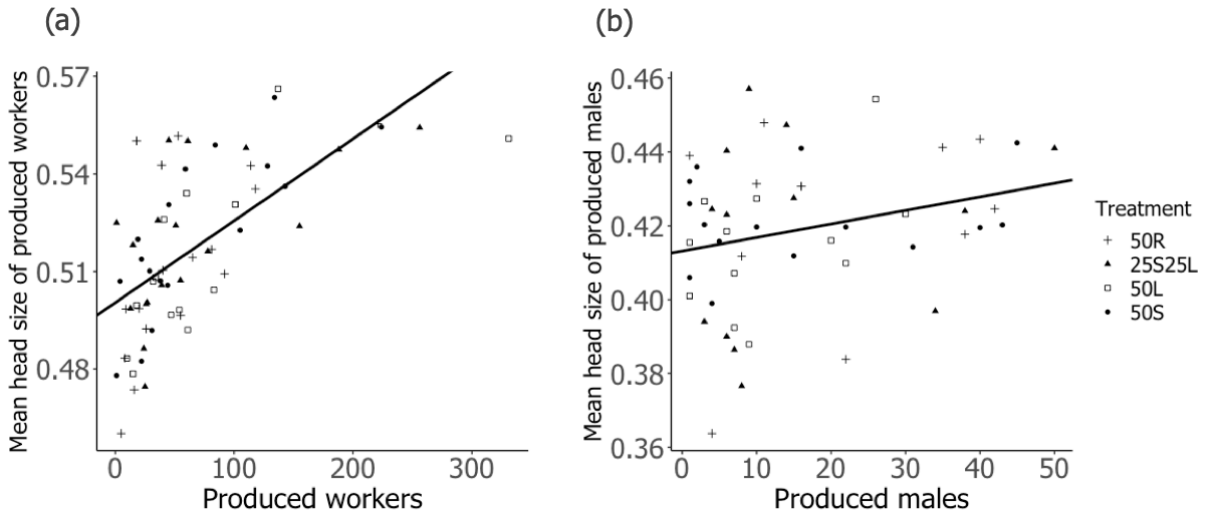
570

571 Table 1: Models and statistics related to colony growth. ‘Minimum model’ means that the
572 predictor was retained in the minimum model selected. A mixed effect model with colony as
573 random factor was used for the size of produced workers in order to control for the colony of
574 origin of workers.

575 Table 2: Models and statistics related to reproductive success. ‘Minimum model’ means that
576 the predictor was retained in the minimum model selected. A mixed effect model with colony
577 as random factor was used for the size of produced males in order to control for the colony of
578 origin of males.







Predictors	Response variables			
	Mortality rate of adult workers	Produced workers	CV of produced workers	Size of produced workers
Treatment	Minimum model $F_{57:66} = 1.94, P = 0.063$	$F_{66:63} = 0.38, P = 0.8$	$F_{63:60} = 0.39, P = 0.8$	$\chi^2_3 = 3.25, P = 0.3$
Initial workforce	Minimum model $F_{57:61} = 2.31, P = 0.069$	Minimum model $F_{66:67} = 51.81, P < 0.001$	$F_{63:62} = 0.02, P = 0.9$	$\chi^2_1 = 0.002, P = 0.9$
Treatment - initial workforce interaction	Minimum model $F_{57:60} = 2.52, P = 0.067$	$F_{66:63} = 0.50, P = 0.7$	$F_{63:59} = 0.43, P = 0.8$	$\chi^2_4 = 4.24, P = 0.4$
Initial larvae	Minimum model $F_{57:61} = 2.28, P = 0.07$	$F_{66:65} = 1.94, P = 0.2$	$F_{63:62} = 0.22, P = 0.6$	Minimum model $\chi^2_1 = 3.79, P = 0.05$
Treatment - initial larvae interaction	Minimum model $F_{57:60} = 2.95, P = 0.04$	$F_{66:62} = 0.55, P = 0.7$	$F_{63:59} = 0.54, P = 0.7$	$\chi^2_3 = 4.42, P = 0.22$
Mortality rate of adult workers		Minimum model $F_{66:67} = 4.44, P = 0.04$	$F_{63:62} = 0.05, P = 0.8$	$\chi^2_1 = 0.49, P = 0.5$
Treatment - mortality rate of adult workers interaction		$F_{66:63} = 0.40, P = 0.7$	$F_{63:59} = 0.33, P = 0.8$	$\chi^2_4 = 4.95, P = 0.3$
Produced workers			$F_{63:62} = 0.26, P = 0.6$	Minimum model $\chi^2_1 = 38.51, P < 0.001$
Treatment - produced workers interaction			$F_{63:59} = 1.01, P = 0.4$	$\chi^2_3 = 3.30, P = 0.3$

Predictors	Response variables			
	Males produced	Sexual investment	CV of produced males	Size of produced males
Treatment	$F_{67:64} = 1.22,$ $P = 0.3$	$F_{65:62} = 0.23,$ $P = 0.9$	$F_{45:42} = 0.32, P =$ 0.8	$\chi^2_3 = 0.22, P = 0.9$
Initial workforce	Minimum model $F_{67:68} = \mathbf{20.11},$ $P < \mathbf{0.001}$	$F_{65:64} < 0.001,$ $P = 0.3$	$F_{45:44} = 0.20, P =$ 0.6	$\chi^2_1 = 0.38, P = 0.5$
Treatment - initial workforce interaction	$F_{67:64} = 1.11,$ $P = 0.3$	$F_{65:61} = 0.39,$ $P = 0.9$	$F_{45:41} = 0.37, P =$ 0.8	$\chi^2_4 = 1.15, P = 0.9$
Initial larvae	$F_{67:66} = 0.19,$ $P = 0.7$	$F_{65:64} < 0.001,$ $P = 0.9$	$F_{45:44} = 2.54, P =$ 0.1	$\chi^2_1 = 0.02, P = 0.9$
Treatment - initial larvae interaction	$F_{67:63} = 1.28,$ $P = 0.3$	$F_{65:61} = 0.24,$ $P = 0.9$	$F_{45:41} = 1.08, P =$ 0.4	$\chi^2_4 = 0.46, P = 0.9$
Adult worker mortality rate	$F_{67:66} = 0.07,$ $P = 0.8$	$F_{65:64} = 0.36,$ $P = 0.3$	$F_{45:44} = 0.08, P =$ 0.8	$\chi^2_1 = 0.72, P = 0.4$
Treatment - adult worker mortality rate interaction	$F_{67:63} = 0.51,$ $P = 0.7$	$F_{65:61} = 0.73,$ $P = 0.7$	$F_{45:41} = 0.08, P =$ 0.9	$\chi^2_4 = 1.78, P = 0.8$
Males produced			$F_{45:44} = 1.32, P =$ 0.3	Minimum model $\chi^2_1 = \mathbf{4.03}, P =$ $\mathbf{0.04}$
Treatment - males produced interaction			$F_{45:41} = 0.43, P =$ 0.8	$\chi^2_3 = 0.12, P = 0.9$

587 **Appendix captions**

588

589 Appendix A1: Worker size diversity within one colony of *Temnothorax nylanderi*. In our
590 experiment, the head width of the smallest worker produced was 0.384mm, that of the largest
591 was 0.661mm.

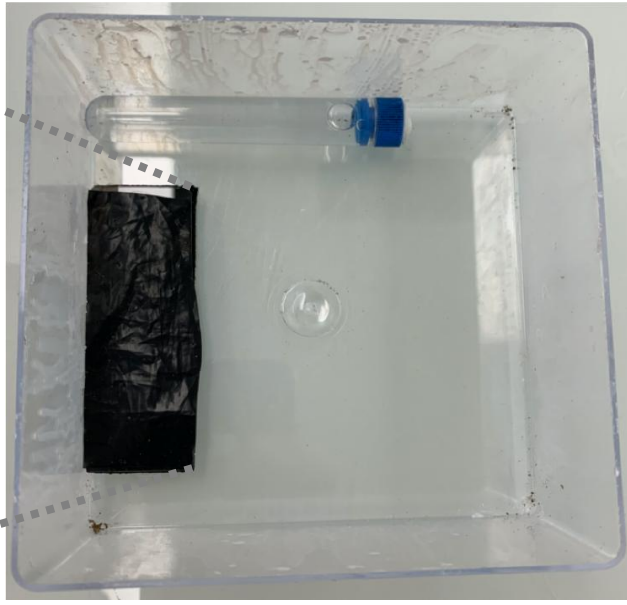
592

593 Appendix A2: Laboratory rearing setup. The nest consisted of two microscope slides
594 separated by a 1-mm auto-adhesive plastic foam with three chambers, covered with black
595 paper for darkness. It was placed in a plastic box (foraging area) with water (tube plugged
596 with cotton) and food (mealworm).

597

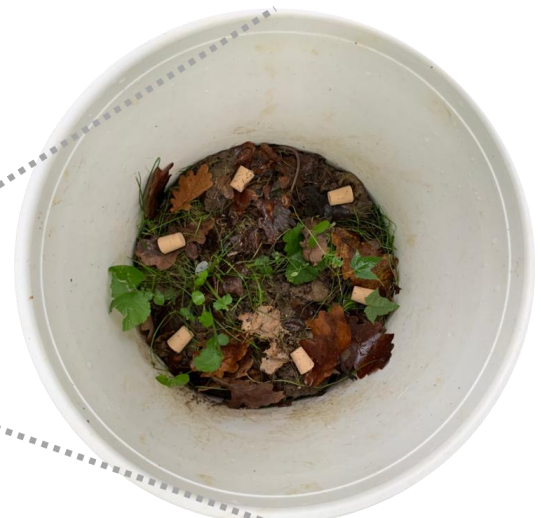
598 Appendix A3: Field rearing setup. Eighty semi-buried buckets were installed in the ‘réserve
599 ornithologique du Bois de Vincennes’. Each bucket contained local soil and was pierced at
600 the bottom for water draining. Six nests (drilled corks) were available for the colony to
601 choose. The foraging area was restricted to the bucket. Cut-off lids are not present on the
602 photos.







608



609

