

1 **Title:** Emergent behavioral organization in heterogeneous groups of a social insect

2

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23 **Author Contributions**

24 YU and DJCK conceived the study. MK, CKT, and CET developed the theoretical approach. YU
25 and DJCK designed the experiments. YU, JS, and VC performed the experiments. YU analyzed
26 the experiments. MK and CKT performed the simulations, and MK, CKT, and CET analyzed the
27 simulation results. MK performed analytical calculations with input from CET. YU, MK, CKT,
28 CET, and DJCK drafted the paper, and all authors provided comments.

29 **This PDF file includes:**

30 Main Text
31 Figures 1 to 5
32 Table 1

33 **Abstract**

34 The composition of social groups has profound effects on their function, from collective decision-
35 making to foraging efficiency. But few social systems afford sufficient control over group
36 composition to precisely quantify its effects on individual and collective behavior. Here we
37 combine experimental and theoretical approaches to study the effect of group composition on
38 individual behavior and division of labor (DOL) in a social insect. Experimentally, we use
39 automated behavioral tracking to monitor 120 colonies of the clonal raider ant, *Ooceraea biroi*,
40 with controlled variation in three key correlates of social insect behavior: genotype, age, and
41 morphology. We find that each of these sources of heterogeneity generates a distinct pattern of
42 behavioral organization, including the amplification or dampening of inherent behavioral
43 differences in colonies with mixed types. Theoretically, we use a well-studied model of DOL to
44 explore potential mechanisms underlying the experimental findings. We find that the simplest
45 implementation of this model, which assumes that heterogeneous individuals differ only in
46 response thresholds, could only partially recapitulate the empirically observed patterns of
47 behavior. However, the full spectrum of observed phenomena was recapitulated by extending the
48 model to incorporate two factors that are biologically meaningful but theoretically rarely
49 considered: variation among workers in task performance efficiency and among larvae in task
50 demand. Our results thus show that different sources of heterogeneity within social groups can
51 generate different, sometimes non-intuitive, behavioral effects, but that relatively simple models
52 can capture these dynamics and thereby begin to elucidate the basic organizational principles of
53 DOL in social insects.

54 **Significance Statement**

55 When individuals interact in an aggregate, many factors that are not known *a priori* affect group
56 dynamics. A social group will therefore show emergent properties that cannot easily be predicted
57 from how its members behave in isolation. This problem is exacerbated in mixed groups, where
58 different individuals have different behavioral tendencies. Here we describe different facets of
59 collective behavioral organization in mixed groups of the clonal raider ant, and show that a
60 simple theoretical model can capture even non-intuitive aspects of the behavioral data. These
61 results begin to reveal the principles underlying emergent behavioral organization in social
62 insects. Importantly, our insights might apply to complex biological systems more generally and
63 be used to help engineer collective behavior in artificial systems.

64 **Main Text**

65

66 **Introduction**

67

68 The study of collective behavior and self-organization is an active area of research across a
69 diversity of fields, from animal movement (1) to robotics (2), from tissue engineering (3) to
70 public health (4), and from voting (5) to conservation (6). The colonies of social insects in
71 particular are striking examples of highly integrated, complex biological systems that can self-
72 regulate without centralized control (7). Consequently, social insects have emerged as powerful
73 systems to study collective behavior and social dynamics, both experimentally and theoretically
74 (8–12). However, few experimental studies have comprehensively measured the influence of
75 group composition—e.g., in age, genotype, or morphology—on collective organization, because
76 the inherent complexity of many social insect colonies renders their composition intractable. This
77 has limited our understanding of how colony composition affects both individual behavior and
78 emergent group-level organization, and constitutes a major hurdle towards a general and
79 comprehensive systems-level description of social insect colonies.

80

81 An emergent colony-level trait that has long been thought to depend on colony composition is
82 division of labor (DOL). DOL describes the non-random variation in task performance among
83 members of a social group (13), and is characterized both by between-individual variation and by
84 individual specialization in task performance. Specifically, DOL has been hypothesized to
85 increase with workforce heterogeneity, based on the observation that individual traits often
86 correlate with individual task allocation (14). For example, workers of different age (15–18),
87 genotype (e.g., patriline (19, 20) or matriline (21)), or morphology (e.g., size (19, 22–24)) can
88 vary in their propensity to engage in tasks such as foraging, nursing, nest construction, or
89 grooming of nestmates.

90

91 Experimentally testing this hypothesis in a systematic manner has proved challenging, even as
92 theory has confirmed that workforce heterogeneity can indeed lead to the emergence of DOL (13,
93 25). One successful theoretical approach relies on the fixed response threshold model (FTM) of
94 task allocation (26, 27). This model assumes that each task has an associated stimulus that signals
95 the colony demand for that task. The magnitude of a given stimulus decreases with the number,
96 efficiency, and/or average time investment of workers performing the corresponding task.

97 Individuals respond to demands based on internal thresholds that reflect their sensitivity to the

98 stimulus and govern their likelihood of performing a task given its stimulus level: the higher the
99 stimulus level for a task relative to an individual's threshold, the more likely it is to begin
100 performing the task (see Materials and Methods for a detailed description). Thus, fixed thresholds
101 provide a simple mechanism by which individuals dynamically allocate efforts to meet colony
102 demands.

103

104 Previous work on the FTM has focused on differences in individual response thresholds as the
105 primary driver of DOL (26, 28–32). In this simple formulation of the FTM, the heterogeneity in
106 behavior is captured via heterogeneity in individual response thresholds drawn from a normal
107 distribution with mean and variance that can be specific to the task and/or the type of individual.
108 Yet, ants can also vary in other traits, for example in the efficiency with which they perform tasks
109 (33–35) or in the average time spent performing a given task (36). Task demand can be similarly
110 variable: for example, foraging activity levels of workers increase with the number of larvae that
111 they have to tend to (37), and larvae of different genotypes develop into adults with different
112 morphologies when cared for by the same workers (38). Thus, the level of demand emanating
113 from the larvae could depend on their number and genotype. Despite this empirical evidence, few
114 theoretical studies of DOL have explored the significance of inter-individual variation in traits
115 other than response thresholds (14, 39).

116

117 Here we combine experimental and theoretical approaches to study the effect of group
118 composition on both individual behavior and colony-level DOL. We use the FTM as a natural
119 starting point, but systematically investigate a suite of parameters that might be associated with
120 different individual traits of interest. To overcome the practical challenges associated with
121 studying complex social systems empirically, we capitalize on the advantages of the clonal raider
122 ant (*Ooceraea biroi*). The unique biology of this species affords unparalleled control over the
123 main aspects of colony composition that are thought to affect individual- and group-level
124 behavior in social insects: genotype, age, and morphology. Specifically, colonies of clonal raider
125 ants are queenless and exclusively composed of workers that reproduce asexually and
126 synchronously, so that all adults within a colony are genetically almost identical and emerge in
127 discrete age cohorts. Furthermore, individuals show variation in ovariole number that is
128 associated with body size and other morphological features (40), making it possible to
129 approximately sort individuals into 'regular workers' (2-3 ovarioles) and 'intercastes' (4-6
130 ovarioles) based on their size (38). Conveniently, workers of different clonal genotypes, age
131 cohorts, and morphologies can be mixed to create functional chimeric colonies (38). Taking

132 advantage of these features, we quantify individual and collective behavior of *O. biroi* in response
133 to precise, independent manipulations of colony composition along three independent axes, in a
134 single system, and under standardized conditions.

135

136

137 **Results**

138

139 **Baseline theoretical predictions of the ‘simple’ FTM with threshold heterogeneity.** To
140 establish baseline predictions in colonies with two types of ants (e.g., of different genotype, age,
141 or morphology), we simulated experimental colonies using the simplest and most commonly-
142 employed formulation of the FTM described above (see also Materials and Methods). Simulated
143 colonies were either pure or mixed with respect to ant type; pure colonies consisted solely of one
144 type of ant or the other, whereas mixed colonies had the two types in equal proportions. The
145 ‘simple’ FTM assumes that the types only differ in mean response threshold. The individual
146 thresholds for each type of ant are drawn from a normal distribution with the corresponding type-
147 specific mean. All other model parameters—task performance efficiency, demand rate, threshold
148 variance—are constant across types. Thus, the only source of heterogeneity in pure colonies was
149 the distribution of individual response thresholds, while in mixed colonies that heterogeneity was
150 compounded by differences in the means of the type-specific distributions. The assumption that
151 some threshold heterogeneity exists even in pure colonies rests on the experimental observation
152 that pure colonies exhibit DOL, yet in the absence of any type of heterogeneity, the FTM cannot
153 produce DOL (32).

154

155 In pure colonies, there is a single normal distribution of individual thresholds for a given task.
156 Because individuals from the lower end of the distribution are more sensitive to the stimulus for
157 that task, they tended to perform that task more often than those from the higher end, resulting in
158 DOL. In mixed colonies, there is a bimodal distribution of thresholds for each task, with the
159 thresholds of the two types clustered around the different modes. This wider distribution of
160 thresholds resulted in more pronounced DOL, i.e., both behavioral variation and specialization
161 were greater in mixed colonies compared to pure colonies (Fig. 1a-b).

162

163 However, all colonies, irrespective of their composition, had the same mean task performance
164 (Fig. 1c). This is because, while colonies may differ in how they allocate workers to tasks (in this
165 case, within mixed colonies, the two ant types differed in their mean task performance because

166 the type with the lower average threshold for a given task took up that task more often than the
167 other type), they must perform the same amount of work overall to satisfy a given demand. Thus,
168 on average, colony members spent the same fraction of time performing each task across pure and
169 mixed colonies.

170

171 In summary, the simple FTM predicted that (P1) regardless of composition, colonies would
172 exhibit the same average task performance, but that (P2) mixed colonies would exhibit higher
173 overall DOL and that (P3) the two types would behave differently from each other in mixed
174 colonies, but not in their respective pure colonies (see Table 1 for a summary of predictions).

175

176 **Effects of individual genotype, age, and morphology on individual behavior in experimental**

177 **colonies.** We experimentally tested these theoretical predictions in replicate experimental
178 colonies that were either pure or mixed with respect to genetic, demographic, and morphological
179 composition, manipulating each factor independently from the others (see Materials and Methods,
180 Table S1). For example, demographically pure colonies contained either only young workers (1
181 month old) or only old workers (3 month old), and mixed colonies contained young and old
182 workers in equal proportions; genotype and morphology were kept constant both within and
183 between these colonies. Similarly, genetically pure colonies contained either only workers of
184 genotype B or of genotype A (see (41) for genotype designations), and mixed colonies contained
185 workers of the two genotypes in equal proportions; age and morphology were kept constant
186 among these colonies. All colonies within an experiment had the same size. Colonies contained 8
187 or 16 workers—fully functional group sizes in the clonal raider ant—and the same number of
188 age-matched larvae hosted in a Petri dish with a plaster floor (see Materials and Methods). The
189 experiment on genetic effects was performed twice, once with larvae of each genotype.

190

191 We used a high-throughput automated tracking system (32) to record and analyze the behavior of
192 all individual ants in 120 experimental colonies. The propensity of each ant to perform extranidal
193 tasks (e.g., foraging, waste disposal) as opposed to intranidal tasks (e.g., nursing) was computed
194 as the two-dimensional root-mean-square deviation (r.m.s.d.) of its spatial coordinates (32) (Fig.
195 2a; see Materials and Methods). The mean r.m.s.d of a group of ants was used as a proxy for their
196 mean performance of extranidal tasks. To quantify colony-level DOL, we calculated behavioral
197 variation and specialization among colony members. Behavioral variation was computed as the
198 standard deviation across r.m.s.d. values of all ants from the same colony. Specialization was

199 computed as the mean correlation between individual r.m.s.d. ranks across consecutive days in
200 the experiment (32).

201

202 We found that workers of genotype B spent more time away from the nest (i.e., had higher mean
203 r.m.s.d.) than workers of genotype A, both across pure colonies and within mixed colonies (Fig.
204 2b-c), suggesting a genetic basis for the propensity to perform extranidal tasks (19–21). Old
205 workers spent more time away from the nest than young workers irrespective of colony
206 demographic composition (Fig. 2d). Thus, *O. biroi* displays the classic form of age polyethism
207 typical of social insects (15–18, 42), whereby older individuals allocate more time to extranidal
208 tasks, and younger individuals spend more time at the nest. Finally, regular workers spent less
209 time at the nest than intercastes in mixed colonies, but not across pure colonies (Fig. 2e). Because
210 the larger body size and higher reproductive potential of intercastes correspond to a more queen-
211 like phenotype, these behavioral differences support empirical data from other systems—
212 including other queenless (43) and clonal (44) ant species—where reproductive potential often
213 negatively correlates with foraging activity. Thus, consistent with existing knowledge, our
214 experiments revealed robust differences in behavior (here, the propensity to perform extranidal
215 tasks) across ant genotypes, age cohorts, and morphological types (Fig. 2). Interestingly,
216 however, our experiments showed that different ant types (genotypes and age cohorts, but not
217 morphologies) can have different mean behaviors between the corresponding two types of pure
218 colonies. This is inconsistent with theoretical prediction (P1) that colonies, irrespective of their
219 composition, should have the same mean behavior (Table 1).

220

221 **Effects of genetic, demographic, and morphological mixing on DOL and individual**
222 **behavior in experimental colonies.** We found that, in general, mixed colonies had higher
223 DOL—measured as behavioral variation (Fig. S1) and specialization (Fig. 3)—than pure
224 colonies. Although not all pairwise comparisons were statistically significant, there was no case
225 where pure colonies had significantly higher DOL than mixed colonies. Thus, each of the three
226 forms of workforce heterogeneity tended to promote DOL, consistent with prediction (P2) (see
227 Table 1).

228

229 We next assessed the outcome of mixing individuals with different behavioral tendencies on
230 individual behavior. Consider two types of individuals, X and Y. Let X_k and Y_k be the mean
231 behavior of types X and Y, respectively, in pure ($k = p$) or mixed ($k = m$) colonies. We assume
232 that $Y_p > X_p$ and $Y_m > X_m$, to reflect our observation that the type with higher r.m.s.d. in pure

233 colonies always also had higher r.m.s.d in mixed colonies. Given this assumption, mixing could,
234 in principle, have one of three possible outcomes on individual behavior:

235

- 236 1) No effect of mixing on individual behavior: the mean behavioral difference between
237 types across pure colonies is the same as the mean behavioral difference between types
238 within mixed colonies, so that $Y_p - X_p = Y_m - X_m$.
- 239 2) Behavioral ‘contagion’: individuals of different types become behaviorally more similar
240 on average to each other when mixed, so that $Y_p - X_p > Y_m - X_m$; and
- 241 3) Behavioral ‘amplification’: individuals of different types become behaviorally more
242 different on average from each other when mixed, so that $Y_p - X_p < Y_m - X_m$.

243

244 The simple FTM predicted that the two different types will differ in mean behavior when mixed,
245 but not when in pure colonies (P3) (Fig. 1c), i.e., that behavioral amplification should always be
246 observed. However, in contrast to this theoretical prediction, all three outcomes were observed
247 experimentally: genetic mixing resulted in behavioral contagion (Fig. 2b-c; Student’s *t*-test: $t =$
248 $3.86, p = 0.002$ in colonies with A brood, $t = 2.62, p = 0.02$ in colonies with B brood);
249 demographic mixing had no effect on individual behavior (Fig. 2d; $t = -1.50, p = 0.16$); and
250 morphological mixing resulted in behavioral amplification (Fig. 2e; $t = -2.44, p = 0.02$).

251

252 We further investigated whether mixing had an asymmetric effect on the two ant types, i.e.,
253 whether it affected one type more than the other, so that the magnitude of change in type-specific
254 behavior between pure and mixed colonies was different across the two ant types (i.e. $|X_m - X_p| \neq$
255 $|Y_m - Y_p|$). Testing this hypothesis, we found evidence for asymmetric behavioral contagion in
256 genetically mixed colonies with A brood (Fig. 2b), where mixing affected the behavior of A
257 workers (by increasing their extranidal activity) more than it affected the behavior of B workers
258 (*t*-test $|A_m - A_p|$ vs. $|B_m - B_p|$: $t = 3.86, p = 0.0024$). All other scenarios studied displayed
259 symmetric effects of mixing on individual behavior (Fig. 2c: $|A_m - A_p|$ vs. $|B_m - B_p|$: $t = -0.94, p =$
260 0.37 ; Fig. 2d, $|Young_m - Young_p|$ vs. $|Old_m - Old_p|$: $t = -1.02, p = 0.33$, Fig. 2e, $|Regular Worker_m -$
261 $Regular Worker_p|$ vs. $|Intercaste_m - Intercaste_p|$: $t = 0.68, p = 0.50$).

262

263 Thus, both the direction and the magnitude of change in individual behavior between pure and
264 mixed colonies depended on the specific source of workforce heterogeneity.

265 **Theoretical predictions of the extended FTM.** The predictions of the simple FTM only partially
266 captured the patterns observed in the experimental colonies (Table 1). Thus, differences in mean

267 threshold alone were insufficient to explain the observed data, suggesting the need to consider
268 other biologically realistic sources of heterogeneity in the model.

269

270 Much like assuming that types differ solely in their threshold means, assuming that types differ
271 only in threshold variance or duration of task performance failed to capture the experimentally
272 observed difference in mean behavior between pure colonies (Fig. S2a-b). However, between-
273 type differences in task performance efficiency alone did reproduce this difference. In fact, if the
274 demand was the same for both tasks, differences in task efficiency were necessary for such a
275 pattern to emerge (SI Appendix).

276

277 When types differed only in task performance efficiency, we further found behavioral contagion
278 in mixed colonies, i.e., the types behaved more similarly to each other when mixed. Critically, the
279 asymmetry of this contagion depended on the magnitude of the task demand. If the task demand
280 was not too high, so that both types could keep up with the demand in their pure colonies, then
281 the contagion was always downward (Fig. 4a; analytical results in SI Appendix), i.e., the mixed
282 colony, on average, behaved more like the more efficient type. If, on the other hand, the task
283 demand was so high that the less efficient type could not keep up with task demand in its pure
284 colony, then the contagion could, for certain parameter combinations, be upward (Fig. 4b), i.e.,
285 the mixed colony behaved on average more like the less efficient type. Hence, if in addition to
286 differences in task efficiency we also assumed between-type differences in task demand (to
287 reflect possible differences in the intensity of task demand stemming from larvae of different
288 genotypes), we were able to qualitatively recapitulate the asymmetric behavioral contagion
289 observed in genetically mixed colonies. Holding all else fixed, differences in task efficiency
290 guaranteed behavioral contagion; the magnitude of task demand modulated the asymmetry of this
291 contagion, i.e., whether mixed colonies on average behaved more like the more or less efficient
292 type.

293

294 Although the combination of between-type differences in efficiency and demand successfully
295 recapitulated the observed behavioral contagion, it failed to capture the other observed effects of
296 mixing on individual behavior, notably instances where mixing had no effect on behavior, or
297 where it resulted in behavioral amplification. If we instead combined the between-type
298 differences in task efficiency with between-type differences in mean threshold (to reflect possible
299 between-type differences in the intrinsic propensity to perform tasks), we were able to
300 qualitatively recapitulate both the effects of demographic mixing (no effect of mixing on type-

301 specific behavior; Fig. 4c) and the effects of morphological mixing (behavioral amplification in
302 mixed colonies; Figs. 4d, S3). Whether we recapitulated the former or the latter depended on the
303 magnitude of the difference in mean thresholds: a larger difference caused the types to
304 differentiate their behavior more strongly in mixed colonies, leading to the latter; a smaller
305 difference dampened this effect, leading to the former.

306

307 In general, the model robustly produced a spectrum of patterns, from behavioral contagion to
308 amplification, across a large parameter space (Fig. 4e). Thus, incorporating additional,
309 biologically realistic sources of heterogeneity into the model led to predictions that qualitatively
310 mirrored the range of empirically observed behavioral patterns, namely: (P4) pure colonies of
311 different ant types can differ from each other and from the mixed colonies in mean behavior, and
312 (P5) mixing two types of ants can lead to behavioral contagion, amplification, or neither (Table
313 1). Moreover, these extensions preserved prediction P2 (to the extent observed in the
314 experimental data), that mixed colonies tend to have higher DOL than pure colonies (Figs. S4,
315 S5).

316

317 **Theoretical predictions for mean task performance in non-1:1 mixes.** Despite its simplicity,
318 the fixed threshold framework demonstrated remarkable explanatory power in both pure and
319 mixed colonies. Given this success, we used the extended FTM to further explore expected
320 patterns of task allocation in colonies with different ratios of ant types. We focused on the four
321 parameter combinations in Fig. 4 because our analysis showed that they collectively captured all
322 of the patterns observed in the experiments. For each parameter combination, we investigated
323 how the mean task performance of colonies changed as we varied the ratio of the two ant types.

324

325 Simulations predicted a striking range of patterns. For the parameter combination that produced
326 no effect in the mixed colonies with equal proportions of the two ant types ('1:1 mixes'), the
327 model produced an approximately linear relationship between mean task performance and the
328 ratio of ant types (Fig. 5a). In all other cases, the mean task performance depended nonlinearly on
329 the ratio of the types. However, the shape of the nonlinear curve differed among the cases. In the
330 cases corresponding to behavioral contagion in the 1:1 mixes, the relationship followed a convex
331 decreasing function, so long as there were enough individuals of the more efficient type such that
332 the colony could keep up with the demand (Fig. 5b; analytical results in SI Appendix); otherwise
333 the colony performed the tasks at a fixed maximum capacity that depended only on the average
334 task duration (Fig. 5c). In the case corresponding to behavioral amplification, the relationship

335 followed a concave decreasing function (Fig. 5d). Hence, despite one type being more efficient
336 than the other in all cases considered, replacing an individual of the former type with one of the
337 latter type would lead to qualitatively different outcomes depending on the between-type
338 differences in mean threshold.

339

340 Regardless of the case studied, the ratio of the types did not alter the qualitative effect of mixing
341 on individual behavior (behavioral contagion, amplification, or no effect); for example, the case
342 that led to behavioral amplification in 1:1 mixes predicted behavioral amplification for all non-
343 1:1 mixes tested (Fig. 5d).

344

345

346 **Discussion**

347

348 By manipulating social group composition along three different axes, we found that the effects of
349 group heterogeneity on behavioral organization vary qualitatively depending on the specific
350 factor under consideration. When ants of two different genotypes, ages, or morphologies were
351 mixed, the inherent behavioral differences between each pair of types were dampened,
352 unaffected, or amplified, respectively. The fact that various sources of heterogeneity that
353 naturally exist in animal groups can have different, and possibly opposing, effects on collective
354 organization underscores the importance of independently considering and controlling them. In
355 nature, as in many experiments, all the factors studied here (larval and worker genotype, age, and
356 morphology), as well as other effects (e.g., environmental conditions, resource availability) will
357 play out simultaneously and in largely intractable ways. Being able to break this complexity
358 down experimentally to study each effect separately and under standardized conditions is
359 unprecedented and provides new insight into the basic organizing principles of behavior in social
360 groups.

361

362 The experimental literature on DOL in social insects has historically attributed most inter-
363 individual variation in behavior to variation in response thresholds. Our combined empirical and
364 theoretical analyses, however, suggest that this is only part of the story. Indeed, we found that the
365 simplest and most common implementation of the FTM, which assumes that individuals vary in
366 response thresholds alone, only explained part of the empirically observed patterns of behavior.
367 However, the full spectrum of observed phenomena could be qualitatively recapitulated by
368 extending the model to incorporate heterogeneity in two additional factors: task performance

369 efficiency and task demand. Both are empirically documented (33–35, 45, 46) but theoretically
370 rarely considered.

371

372 Between-type differences in threshold and in task efficiency alone—two sources of heterogeneity
373 with opposing effects on behavioral organization—were sufficient to recapitulate the core of our
374 empirical results. Between-type differences in threshold led to behavioral amplification, making
375 ant types behaviorally more different when mixed than when separated, as is known from
376 previous theoretical work on the FTM (26). In contrast, between-type differences in task
377 efficiency led to behavioral contagion, making ant types behaviorally more similar when mixed
378 than when separated. Our theoretical analysis suggests that the relative strengths of these two
379 sources of heterogeneity might vary with colony composition. In our experiments, varying colony
380 *morphological* composition produced behavioral patterns that were theoretically recapitulated
381 under a relatively strong effect of between-type differences in threshold and a relatively weak
382 effect of differences in efficiency. In contrast, the behavioral patterns observed under varied
383 *genetic* composition matched the theoretical predictions for the case in which differences in
384 efficiency have a relatively stronger effect. Manipulating *demographic* composition corresponded
385 to an intermediate scenario in which the two opposing forces seemed to balance each other out.
386 While both threshold (47–49) and efficiency (33–35) are known to vary with various individual
387 traits in social insects, their relative contributions to age-, genotype- and morphology-based
388 behavioral variation remain poorly understood and deserve further investigation.

389

390 A third source of heterogeneity, task demand, was necessary to recapitulate the asymmetry in
391 behavioral contagion (i.e., whether workers in mixed colonies behaved more like one or the other
392 type of workers in pure colonies). Empirically, whether a colony composed of two ant genotypes
393 behaved more like one genotype or the other depended on the genotype of the larvae reared.
394 Coupled with the theoretical analysis, these results suggest that the differences in brood genotype
395 could be a source of differences in task demand. This points to the brood as an important player
396 in the regulation of task allocation, at least for tasks associated with brood care, such as foraging
397 and nursing (50). That the brood can influence colony-level traits has been shown in several
398 social insects where larvae solicit food from workers via chemical (51–53) or behavioral (54, 55)
399 cues that affect worker physiology (52, 56) and behavior (e.g., foraging (57), feeding (58)).
400 However, the effect of larvae on the allocation of tasks across individual workers remains elusive
401 in many social insects due to the challenges associated with measuring individual behavior in
402 groups and precisely controlling brood demand. By taking advantage of automated tracking and

403 the unique biology of the clonal raider ant, our study overcomes these challenges and advances
404 our understanding of larval factors that affect task allocation: we suggest that brood demand and
405 its effects on task allocation depend not only on the presence and number of larvae (37, 59), but
406 also on larval genotype. These results also provide insights into previous cross-fostering
407 experiments that revealed that interactions between worker and brood genotypes have non-linear
408 effects on brood development (into intercastes vs. regular workers) (38). Our work suggests that
409 these effects might arise, at least in part, because different larval genotypes signal different levels
410 of demand—and thereby differ in the magnitude of their effect on worker behavior—while
411 different worker genotypes differ in their behavioral response to a given level of larval demand.
412 For example, if different larval genotypes solicit food at different rates and different worker
413 genotypes respond differently (e.g., via foraging thresholds or efficiency) to such differences in
414 demand, the interaction between genetically-based larval demand and worker behavioral
415 responses may result in differences in larval nutrition. Such differences may, in turn, lead to the
416 previously reported shifts in larval development and, therefore, adult phenotype (38).

417
418 Overall, these findings demonstrate that, despite its simplicity, the FTM has remarkable
419 versatility in recapitulating a broad range of experimental outcomes, while still operating under
420 biologically plausible assumptions. It is important to note, however, that while the behaviors
421 observed are robust and generic—i.e., the parameters chosen to illustrate the versatility of the
422 FTM are representative of large regions of parameter space—little is known about what
423 parameter values might actually correspond to the different experimental types. Nevertheless,
424 even in the absence of such experimental measurements, the model provides a useful starting
425 point to generate testable predictions for increasingly complex colony compositions in the clonal
426 raider ant and possibly other social insects.

427
428 Our findings add to the growing literature on the role of individual heterogeneity in the collective
429 behavior of complex biological (e.g., schools of fish, neurons in a brain, pathogen strains sharing
430 a host, etc.) and artificial (e.g., heterogeneous robot swarms, synthetic microbial communities,
431 etc.) systems. Much like colonies of the clonal raider ant, these systems exhibit patterns that can
432 be interpreted as behavioral convergence (60–64), divergence (65), and non-linear effects of
433 mixing on group-level phenotypes (66–68). In turn, these patterns affect important processes such
434 as collective decision-making (5), the transmission and evolution of disease (69, 70), and the
435 evolution of cooperative behavior (71, 72). While different variants of threshold-based models
436 have been employed to study several of these systems (73–76), we still lack a unified theoretical

437 framework to understand the consequences of individual differences on collective dynamics (77).
438 Thus, a comparative approach to the study of the basic organizing principles of heterogeneous
439 systems across scales constitutes an important next step towards understanding the behavior of
440 complex biological systems.

441

442

443 **Materials and Methods**

444

445 **Experimental design.** Four experiments were performed to investigate the effect of genetic
446 composition (2 experiments differing in the brood genotype used), demographic composition (1
447 experiment), and morphological composition (1 experiment). Each experiment comprised three
448 treatments (2 with pure colonies, 1 with mixed colonies). All colonies within one experiment
449 were monitored in parallel, but the different experiments were performed separately.

450

451 Experimental colonies were composed of workers of the desired age, genotype and morphology
452 (Table S1), as well as larvae, housed in airtight Petri dishes 5 cm in diameter (corresponding to
453 about 25 ant body-lengths) with a plaster of Paris floor. To control individual genotype, clonally
454 related workers were sourced from the same stock colony. We used two commonly used
455 genotypes, A and B (32, 38, 78). To control individual age, workers were sourced from a single
456 age cohort from the same stock colony. Owing to the synchronized reproduction of *O. biroi*, all
457 age-matched workers collected this way had eclosed within a day of each other. To control
458 individual morphology, age-matched regular workers and intercastes from the same stock colony
459 were screened based on body size (small or large) and the absence or presence of vestigial eyes,
460 respectively. From the time they were collected (1–3 days after eclosion) until the start of
461 experiments, workers of a given type were kept as a group. All workers were tagged with color
462 marks on the thorax and gaster using oil-paint markers. Experimental colonies contained 16
463 (genetic composition and demographic composition experiments) or 8 (morphological
464 composition experiment) workers and a matching number of age-matched larvae (4-5 days old).
465 This 1:1 larvae-to-workers ratio corresponds to the estimated ratio found in a typical laboratory
466 stock colony in the brood-care phase. We used 8 (genetic composition and demographic
467 composition experiments) or 16 (morphological composition experiment) replicate colonies were
468 set up for each group composition, for a total of 120 colonies.

469

470 The experiments took place in a climate room at 25 °C and 75% relative humidity under constant
471 light (*O. biroi* is blind and its behavior is not affected by light). Every 3 days, we cleaned and
472 watered the plaster, and added one prey item (live pupae of fire ant minor workers) per live larva
473 at a random location within the Petri dish.

474

475 Behavioral data acquisition and analyses were performed as in (32). Software for image analysis
476 is available at <https://doi.org/10.5281/zenodo.1211644>.

477

478 **Behavioral data analyses.** *O. biroi* colonies switch between reproductive phases, in which all
479 workers stay in the nest and lay eggs, and brood-care phases, in which workers nurse the larvae in
480 the nest but also leave the nest to forage, explore, or dispose of waste. For each colony,
481 behavioral analyses were restricted to the brood-care phase, which started at the beginning of the
482 experiment and ended when all larvae had either reached the non-feeding pre-pupal stage or died.

483

484 The spatial distribution of each ant throughout the brood-care phase was quantified as the two-
485 dimensional root-mean-square deviation:

486
$$\text{r. m. s. d.} = \sqrt{\frac{\sum_i ((x_i - \bar{x})^2 + (y_i - \bar{y})^2)}{n}}$$

487 in which x_i and y_i are the coordinates of the focal ant in frame i , and \bar{x} and \bar{y} are the coordinates
488 of the center of mass of the focal ant's overall spatial distribution in the brood-care phase, and n
489 is the number of frames in which the focal ant was detected. The r.m.s.d. is bounded between 0
490 and r , the radius of the Petri dish. Workers that spend a lot of time at the nest with the brood (e.g.,
491 nursing the larvae) and little time performing extranidal tasks (foraging or waste disposal) have
492 low r.m.s.d. values, whereas workers that spend more time away from the brood have higher
493 r.m.s.d. values.

494

495 For each colony, mean behavior was computed as the average of individual r.m.s.d. values, and
496 behavioral variation was computed as the standard deviation of individual r.m.s.d. values. Both
497 metrics were then averaged across replicate colonies for each treatment.

498

499 To quantify specialization, we use a metric appropriate for use on continuous behavioral data
500 (r.m.s.d.). Specialization was defined for each colony as the Spearman correlation coefficient
501 between individual r.m.s.d. ranks on consecutive days, averaged over the brood-care phase. Mean

502 rank-correlation coefficients were then compared across treatments. For all behavioral analyses,
503 ants were excluded from the dataset if they were detected in less than 30% of the frames acquired
504 within the considered time frame (brood-care phase or day); for ants that died during the brood-
505 care phase, the considered time frame was the portion of the brood-care phase preceding death.

506

507 **Statistical analyses.** Statistical analyses were performed in R (79). Analyses were performed
508 separately for each of the four experiments. As the experiments were performed at different times
509 using different cohorts of ants, we cannot rule out “batch” effects and therefore avoid any
510 statistical analyses comparing treatments across experiments.

511

512 *Effects of individual attributes traits on behavior.* The effects of colony composition (pure,
513 mixed), individual attributes (genotypes A vs. B, Young vs. Old, or Regular worker vs.
514 Intercaste), and their interaction, on individual behavior (individual r.m.s.d.) were investigated
515 using linear mixed effects (LME, function *lmer* of package *lme4*) models with colony as a
516 random factor. If a significant interaction between colony composition and individual attributes
517 was detected, we used a second LME model with a four-level independent fixed variable
518 combining colony composition and individual attributes (X_p , Y_p , X_m and Y_m , where X_k and Y_k are
519 the mean behavior of ant types X and Y, respectively, in pure ($k=p$) or mixed colonies ($k=m$)),
520 followed by a Tukey posthoc test with Bonferroni-Holm correction (function *glht* of package
521 *multcomp*) for the following planned comparisons: X_p vs. X_m , Y_p vs. Y_m , X_p vs. Y_p , and X_m vs.
522 Y_m . When needed, response variables were transformed to satisfy model assumptions.

523

524 *Effects of genetic, demographic, and morphological mixing on DOL.* The effects of the treatment
525 (a 3-level variable: X, Y, and mixed) on division of labor (behavioral variation, specialization)
526 were investigated using generalized linear models (GLM), followed by Tukey posthoc tests with
527 Bonferroni-Holm correction for all three pairwise comparisons.

528

529 *Effects of genetic, demographic, and morphological mixing on individual behavior.* To assess
530 whether type-specific behavior was affected by colony composition, we compared the difference
531 in mean behavior (mean r.m.s.d.) between types across pure colonies to the difference in mean
532 behavior between the same types within mixed colonies (i.e., $Y_p - X_p$ vs. $Y_m - X_m$, where $Y_p > X_p$
533 and $Y_m > X_m$), using unpaired t-tests, after verifying assumptions of normality. We further tested
534 whether the amplitude of the effect differed across types by comparing the magnitude of change

535 in type-specific behavior between pure and mixed colonies across the two ant types (i.e. $|X_m - X_p|$
 536 $\neq |Y_m - Y_p|$) with unpaired t-tests, after verifying assumptions of normality.

537

538 **Theoretical model.** The fixed threshold model (FTM) considers a colony of n individuals, N_X of
 539 which are of type X and N_Y are of type Y ($N_X + N_Y = n$). Types X and Y represent any pair of the
 540 experimentally manipulated sub-colony compositions (i.e., genotypes A and B, Young and Old,
 541 or Regular Workers and Intercastes). Without loss of generality, we assume that individuals 1, ...,
 542 N_X are of type X and individuals $N_X + 1, \dots, n$ are of type Y. The colony must perform m tasks; for
 543 consistency with the experimental approach, we assume that there are two tasks ($m = 2$). At a
 544 given time step, an individual can be either performing one of the m tasks (active) or not
 545 performing any (inactive). The *task state* of individual i at time t is given by the binary variable
 546 $x_{ij,t}$: if individual i is active and performing task j at time t , then $x_{ij,t} = 1$ and $x_{ij',t} = 0$ for all $j' \neq j$; if
 547 individual i is inactive and in its rest state, then $x_{ij,t} = 0$ for all j . The task state of the colony at
 548 time t is then given by the n -by- m binary matrix $Q_t = [x_{ij,t}]$.

549

550 The FTM further assumes that each task j has an associated *stimulus* $s_{j,t}$. This stimulus signals the
 551 group-level demand for task j and changes depending on both the rate at which the demand
 552 increases (e.g., the demand for foraging increases due to increased hunger in the colony) and the
 553 number of individuals performing the task (e.g., the demand for foraging decreases as workers go
 554 out and find food). Mathematically, the change in stimulus $s_{j,t}$ is governed by Eq. (1):

$$555 \quad s_{j,t+1} = s_{j,t} + \delta_j - \frac{\alpha_j^X n_{j,t}^X + \alpha_j^Y n_{j,t}^Y}{n}, \quad (1)$$

556 where δ_j is the task-specific demand rate, taken to be constant over time; α_j^X and α_j^Y are the task-
 557 specific performance efficiency of type X and Y individuals, respectively; and $n_{j,t}^X = \sum_{i=1}^{N_X} x_{ij,t}$ and
 558 $n_{j,t}^Y = \sum_{i=N_X+1}^n x_{ij,t}$ are the numbers of type X and Y individuals performing task j at time t ,
 559 respectively.

560

561 Each individual i is assumed to have an internal *threshold* for task j , θ_{ij} , drawn at time $t = 0$ from a
 562 normal distribution with mean μ_j and normalized standard deviation σ_j (i.e., expressed as a
 563 fraction of the corresponding mean μ_j). Although thresholds may change over the individuals'
 564 lifetime, they are assumed to be fixed over the timescale of the experiments and, consequently,
 565 over the simulation runs. We refer to μ_j as the *mean task threshold* and to σ_j as the *threshold*
 566 *variance* for task j ; each can be group- and/or task-specific (i.e., $\alpha_j^X, \alpha_j^Y, \sigma_j^X, \sigma_j^Y$).

567

568 At each time step, inactive individuals assess the m task stimuli in a random sequence until they
569 either begin performing a task or have encountered all stimuli without landing on a task. For each
570 encountered stimulus, individual i evaluates whether to perform the task by comparing the
571 stimulus level to its internal threshold. Specifically, given stimulus $s_{j,t}$ and internal threshold θ_{ij} ,
572 individual i commits to performing task j with probability

$$573 \quad P_{ij} = \frac{s_{ij}^{\eta}}{s_{ij}^{\eta} + \theta_{ij}^{\eta}}, \quad (2)$$

574 where parameter η governs the steepness of this response threshold function. The larger the value
575 of η , the more deterministic the behavior; in the limit $\eta \rightarrow \infty$, the response function becomes a
576 step function ($H[s_{j,t} - \theta_{ij}] = 0$ if $\theta_{ij} > s_{j,t}$, 1 if $\theta_{ij} < s_{j,t}$) where H is the Heaviside function). Active
577 individuals spontaneously quit their task with a constant quit probability τ . Active individuals can
578 neither evaluate stimuli nor switch tasks without first quitting their current task.

579

580 Each agent-based simulation lasted $T = 10,000$ time steps. All simulations and the subsequent
581 analyses were conducted in R (79).

582

583

584

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598

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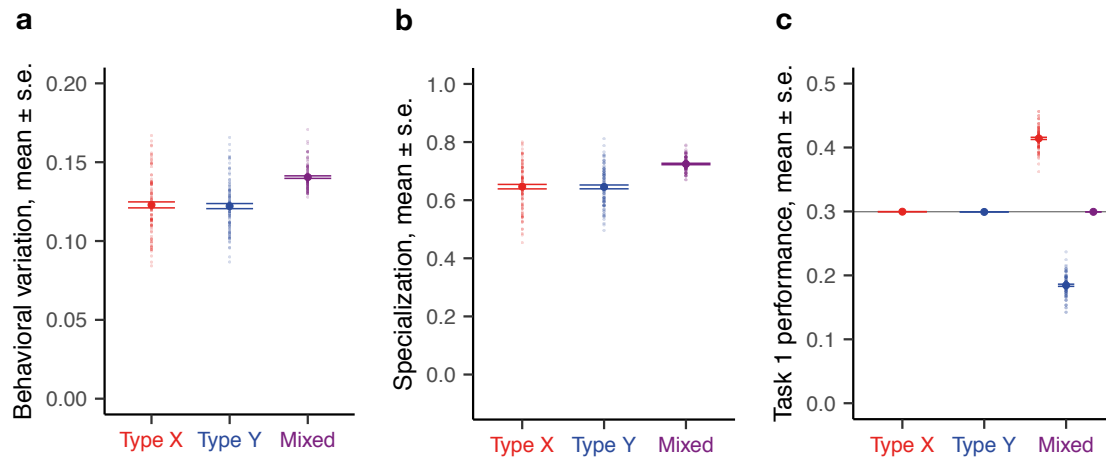
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- 777

778 **Figures and Tables**

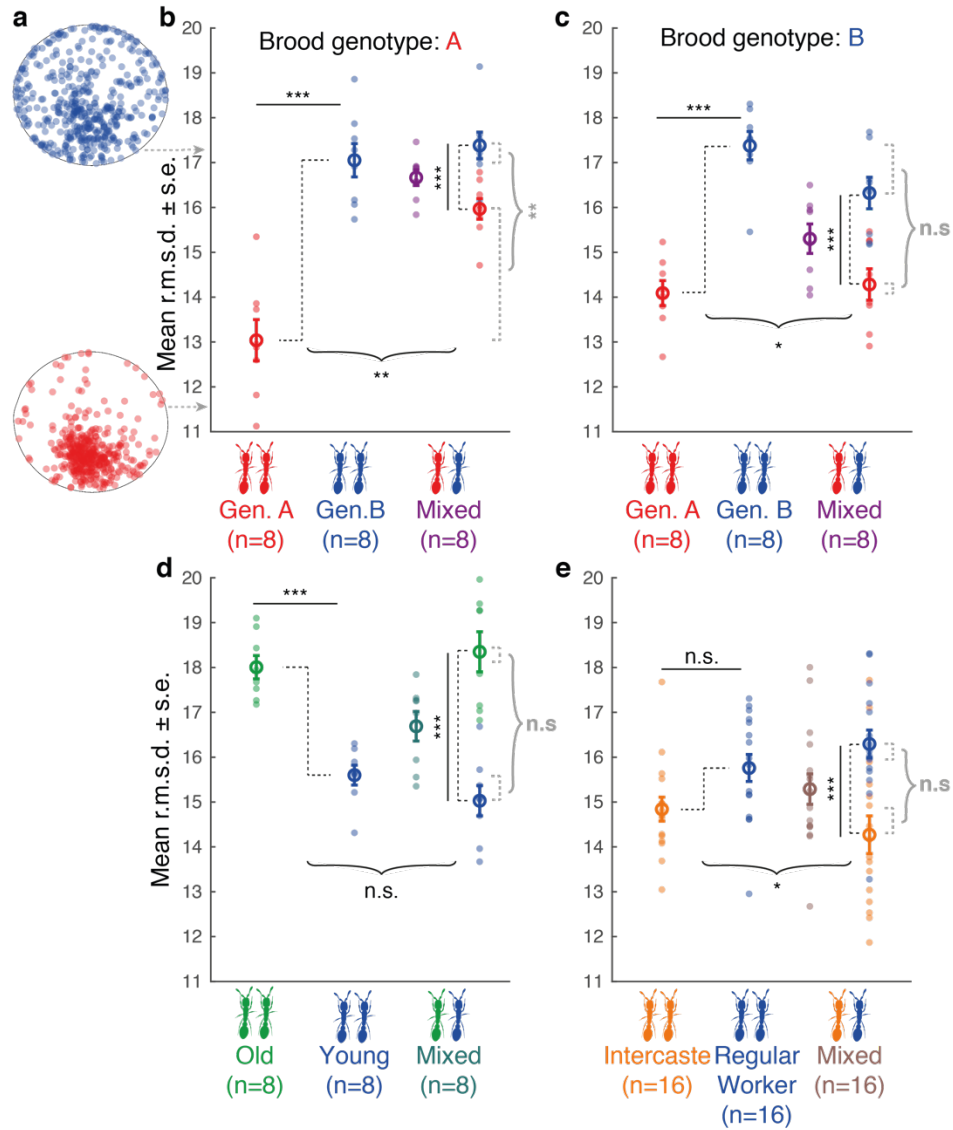
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780

781 **Figure 1. Theoretical predictions of the simple FTM with differences in mean task thresholds only.**

782 One hundred replicates were simulated for each colony composition. Each opaque circle represents an
783 individual replicate colony (colony size 16); each solid circle represents the average value across all
784 replicates for its corresponding colony (or sub-colony) composition. Panels show division of labor
785 (behavioral variation (a), specialization (b)) and task performance frequency (c) as a function of colony
786 composition. Type Y individuals have a higher mean threshold than type X individuals for both tasks ($\mu^X =$
787 $10, \mu^Y = 20$). All other parameters are identical for both types: $\delta = 0.6, \alpha = 2, \sigma = 0.1, \eta = 7, \tau = 0.2$ (see
788 Materials and Methods and Table S2 for parameter definitions).

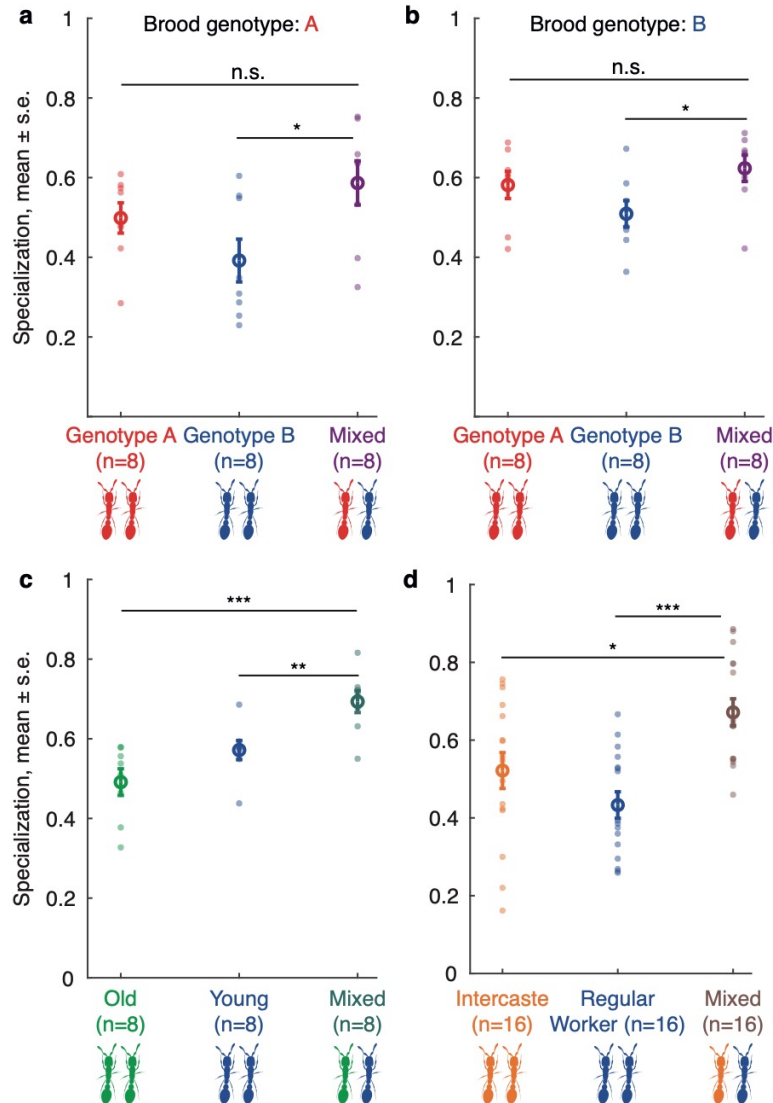


789

790 **Figure 2. Mean r.m.s.d. (a proxy for mean extranidal activity) as a function of colony composition.**

791 Opaque circles represent mean behavior of individuals in replicate colonies (or sub-colonies). Open circles
 792 represent average values across replicate colonies (or sub-colonies). For mixed colonies, data are shown
 793 both as type-specific mean behavior (in type-specific colors) and colony-level mean behavior (in ‘average’
 794 color). Identical colors across panels indicate ants of the same genotype, age, and morphological types.
 795 Sample sizes indicate the number of replicate colonies. Straight solid black brackets represent the effects of
 796 individual traits on behavior (X_p vs. Y_p and X_m vs. Y_m). Black dotted brackets represent the behavioral
 797 differences between types in pure ($Y_p - X_p$) and mixed ($Y_m - X_m$) colonies. Black curly brackets represent the
 798 effect of mixing on inter-type behavioral differences ($Y_p - X_p$ vs. $Y_m - X_m$). Grey curly brackets represent the
 799 asymmetry of the effect of mixing between types ($|X_p - X_m|$ vs. $|Y_p - Y_m|$). **a**: Spatial distribution of two ants
 800 with high (blue; genotype B) and low (red; genotype A) r.m.s.d. from the same colony. Arrows point to the
 801 corresponding r.m.s.d. values. **b**: Behavior as a function of colony genetic composition in colonies with A

802 brood. Colony size 16. GLMM post hoc Tukey tests (B_p vs. A_p : $z = 7.75$, $p = 3.64 \times 10^{-14}$; B_m vs. A_m : $z =$
803 4.61 , $p = 8.06 \times 10^{-06}$) **c:** Behavior as a function of colony genetic composition in colonies with B brood.
804 Colony size 16. (B_p vs. A_p : $z = 7.45$, $p = 2.80 \times 10^{-13}$; B_m vs. A_m : $z = 7.68$, $p = 6.57 \times 10^{-14}$) **d:** Behavior as a
805 function of colony demographic composition. Colony size 16 ($Young_p$ vs. Old_p : $z = -6.05$, $p = 4.39 \times 10^{-09}$;
806 $Young_m$ vs. Old_m : $z = -13.31$, $p < 2 \times 10^{-16}$). **e:** Behavior as a function of colony morphological composition.
807 Colony size 8. ($Regular\ Worker_p$ vs. $Intercaste_p$: $z = 2.14$, $p = 0.10$, $Regular\ Worker_m$ vs. $Intercaste_m$: $z =$
808 8.95 , $p < 2 \times 10^{-16}$). n.s.: non-significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.



809

810 **Figure 3. Specialization (day-to-day rank correlation in r.m.s.d.) as a function of colony**

811 **composition.** Opaque circles represent replicate colonies. Open circles represent average values across

812 replicate colonies. Sample sizes indicate the number of replicate colonies. Identical colors across panels

813 indicate ants of the same genotype, age, and morphological types. **a:** Specialization as a function of colony

814 genetic composition in colonies with A brood. Colony size 16. (GLM post hoc Tukey tests; B_p vs. mixed: z

815 = -2.78, $p = 0.02$; A_p vs. mixed: $z = 1.25$, $p = 0.26$) **b:** Specialization as a function of colony genetic

816 composition in colonies with B brood. Colony size 16. (B_p vs. mixed: $z = -2.41$, $p = 0.047$; A_p vs. mixed: z

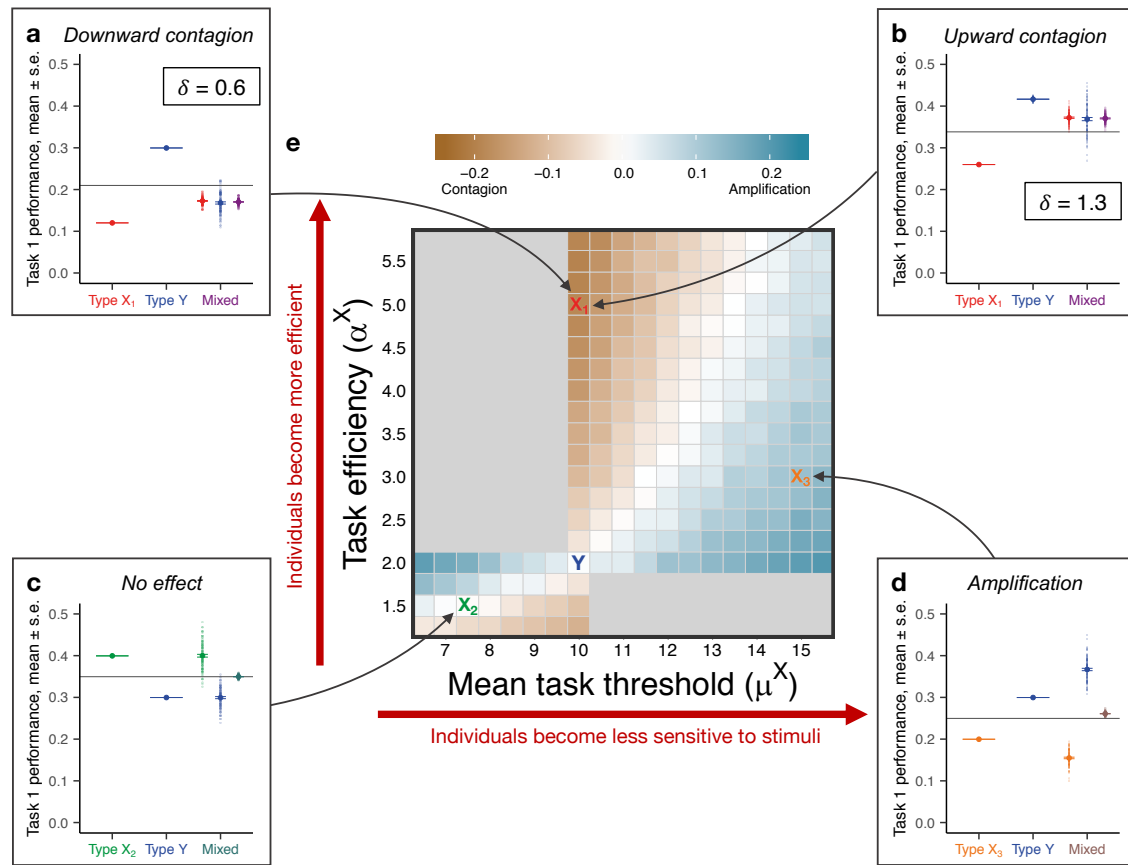
817 = 0.88, $p = 0.38$) **c:** Specialization as a function of colony demographic composition. Colony size 16

818 (Young_p vs. mixed: $z = 3.01$, $p = 0.005$; Old_p vs. mixed: $z = 5.01$, $p = 1.63 \times 10^{-6}$) **d:** Specialization as a

819 function of colony morphological composition. Colony size 8 (Regular Worker_p vs. mixed: $z = -4.35$, $p =$

820 4.05×10^{-5} , Intercaste_p vs. mixed: $z = 2.73$, $p = 0.013$). n.s.: non-significant, *: $p < 0.05$, **: $p < 0.01$, ***: p

821 < 0.001 .



822

823

Figure 4. Theoretical predictions of the FTM on task performance and their robustness. a-d: Task

824

performance frequency as a function of colony composition. One hundred replicates were simulated for

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each colony composition. Each opaque circle represents a replicate colony (colony size 16); each solid

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circle represents the average value across all replicates for its corresponding colony (or sub-colony)

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composition. Horizontal gray lines represent the average value of the pure colonies (first two columns) in

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their respective panels. Identical colors across panels indicate ants of the same types; in particular, the

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parameters for type Y ants are fixed across panels **a-d** ($\mu^Y = 10$, $\alpha^Y = 2$). **a-b:** Differences in task efficiency

830

(α) between types and demand rate (δ) across colonies capture asymmetric behavioral contagion, downward

831

(**a**) and upward (**b**). Larvae are more demanding in **b** ($\delta = 1.3$) than in **a** ($\delta = 0.6$). For a given δ , type X_1 is

832

more efficient than type Y ($\alpha^{X_1} = 5$, $\alpha^Y = 2$). Type Y can keep up with demand when the larvae are less

833

demanding (**a**) but not when they are more demanding (**b**); type X_1 can keep up with the demand in both

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cases. Parameters: $\sigma = 0.1$, $\mu = 10$, $\eta = 7$, $\tau = 0.2$. **c-d:** Between-type differences in task efficiency (α) and

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mean threshold (μ) capture both a lack of effects from mixing (**c**) and behavioral amplification (**d**). In **c**,

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type X_2 is less efficient than type Y ($\alpha^{X_2} = 1.5$, $\alpha^Y = 2$) and has a lower threshold for both tasks ($\mu^{X_2} = 7.5$, μ^Y

837

$= 10$). In **d**, type X_3 is more efficient than type Y ($\alpha^{X_3} = 3$, $\alpha^Y = 2$) and has a higher threshold for both tasks

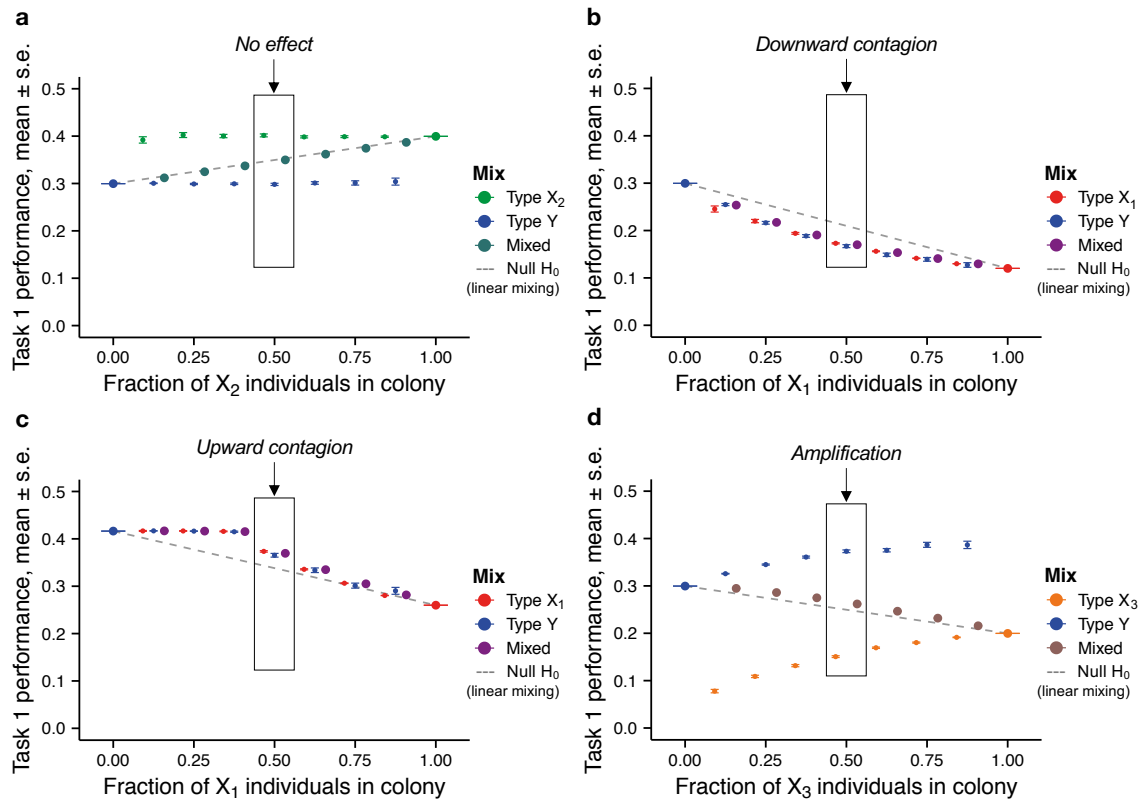
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($\mu^{X_3} = 15$, $\mu^Y = 10$). Parameters: $\sigma = 0.1$, $\eta = 7$, $\delta = 0.6$, $\tau = 0.2$. **e:** Change in between-type relative task

839

performance between mixed and pure colonies (measured as $(Y_m - X_m) - (Y_p - X_p)$) as a function of type X's

840 task efficiency, α^X , and mean task threshold, μ^X . The letters X_1 , X_2 , and X_3 indicate the parameter settings
841 for type X in **a-d**; the blue letter Y indicates the parameter settings for type Y, which are fixed for **a-d** and
842 all grids in **e**. Shades of blue indicate behavioral amplification ($Y_p - X_p < Y_m - X_m$), and shades of brown
843 indicate behavioral contagion ($Y_p - X_p > Y_m - X_m$); light gray indicates regions in which the behavior is
844 undefined according to our definitions of the behavioral patterns, which exclude biologically unrealistic
845 scenarios (see Results). Fifty replicates were simulated for each parameter combination. Parameters: $\eta = 7$,
846 $\sigma = 0.1$, $\tau = 0.2$.





847

848 **Figure 5. Predictions of the FTM for non-1:1 mixes.** Colonies of size 16 with varying ratios of X and Y
849 individuals were simulated under different conditions of threshold values, task-performance efficiency, and
850 task demand. One hundred replicates were simulated for each colony composition. Each large circle
851 represents the mean for that mix of X and Y individuals, while the neighboring smaller circles represent the
852 means of X and Y individuals, respectively, within that mix. The dashed lines indicate the null hypothesis
853 of linear behavioral effects of mixing types. The boxes highlight the behavioral patterns that characterize
854 the 1:1-mixes, and their labels indicate correspondence with panels in Fig. 4 (a with Fig. 4c, b with Fig. 4a,
855 c with Fig. 4b, and d with Fig. 4d). Parameters for each type (X₁, X₂, X₃, Y) are identical to those of the
856 corresponding type in Fig. 4. **a:** X₂ individuals have a lower mean task threshold and are less efficient than
857 Y individuals. **b:** X₁ individuals are more efficient than Y individuals. **c:** X₁ individuals are more efficient
858 than Y individuals, but task demand is high. **d:** X₃ individuals have a much higher mean task threshold than
859 Y individuals and are more efficient.

860 **Table 1. Summary of theoretical results.** Theoretical predictions of the simple FTM (top row) and
 861 extended FTM (bottom row) for pure and mixed colonies, as well as the pattern of behavioral change
 862 observed between them. Text in italic highlights key differences in model predictions. Colors indicate
 863 agreement (light green) or disagreement (light red) with experiments.

		Pure colonies		Mixed colonies		Behavioral change from pure to mixed colonies
		Mean task performance	Division of labor (DOL)	Mean task performance	Division of labor (DOL)	
Type of Fixed Threshold Model (FTM)	Simple FTM (with variation in mean response threshold only)	<i>Identical</i> across colonies of different ant types (P1)	Exhibits DOL	<i>Identical</i> to pure colonies (P1)	Higher DOL than in pure colonies (P2)	Behavioral <i>amplification only</i> (P3)
	Extended FTM (with variation in other biologically relevant params)	<i>Different</i> across colonies of different ant types (P4)	Exhibits DOL	<i>Different</i> from pure colonies (P4)	Higher DOL than in pure colonies (P2)	Behavioral <i>contagion, amplification, or neither</i> (P5)

 = experiments **agree** with prediction
 = experiments **disagree** with prediction

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