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

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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

Abstract

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

Significance Statement

When individuals interact in an aggregate, many factors that are not known *a priori* affect group dynamics. A social group will therefore show emergent properties that cannot easily be predicted from how its members behave in isolation. This problem is exacerbated in mixed groups, where different individuals have different behavioral tendencies. Here we describe different facets of collective behavioral organization in mixed groups of the clonal raider ant, and show that a simple theoretical model can capture even non-intuitive aspects of the behavioral data. These results begin to reveal the principles underlying emergent behavioral organization in social insects. Importantly, our insights might apply to complex biological systems more generally and 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., patrilines (19, 20) or matrilines (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

7

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

13

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

14

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

15

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

16

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 $N_X + 1$, …, 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
$$s_{j,t+1} = s_{j,t} + \delta_j - \frac{\alpha_j^X n_{j,t}^X + \alpha_j^Y n_{j,t}^Y}{n},\tag{1}$$

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., α_j^X, α_j^Y, σ_j^X, σ_j^Y).

17

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
$$P_{ij} = \frac{s_{ij}^{\eta}}{s_{ij}^{\eta}+\theta_{ij}^{\eta}}, \tag{2}$$

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 $\eta \to \infty$, 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

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Figures and Tables
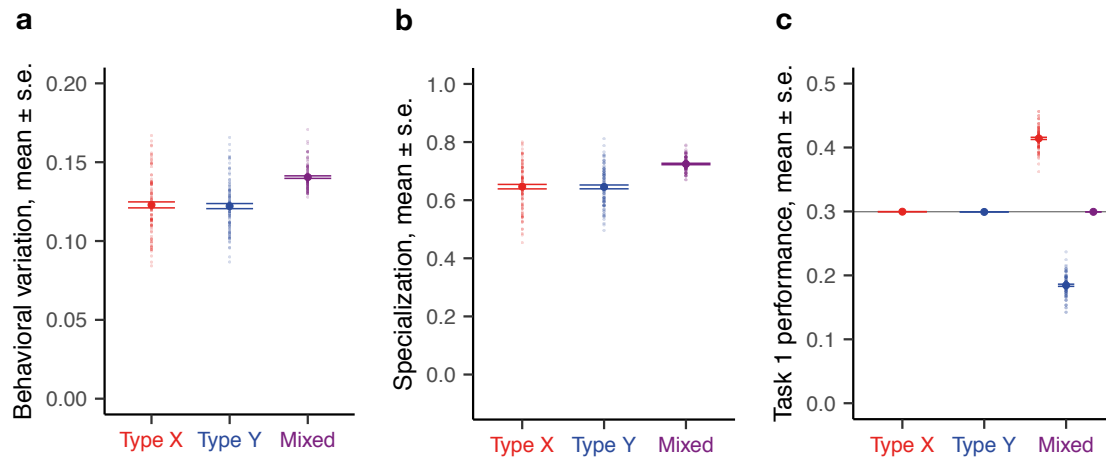
Figure 1. Theoretical predictions of the simple FTM with differences in mean task thresholds only.
One hundred replicates were simulated for each colony composition. Each opaque circle represents an
individual replicate colony (colony size 16); each solid circle represents the average value across all
replicates for its corresponding colony (or sub-colony) composition. Panels show division of labor
(behavioral variation (**a**), specialization (**b**)) and task performance frequency (**c**) as a function of colony
composition. Type Y individuals have a higher mean threshold than type X individuals for both tasks ($\mu^X =$
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
Materials and Methods and Table S2 for parameter definitions).

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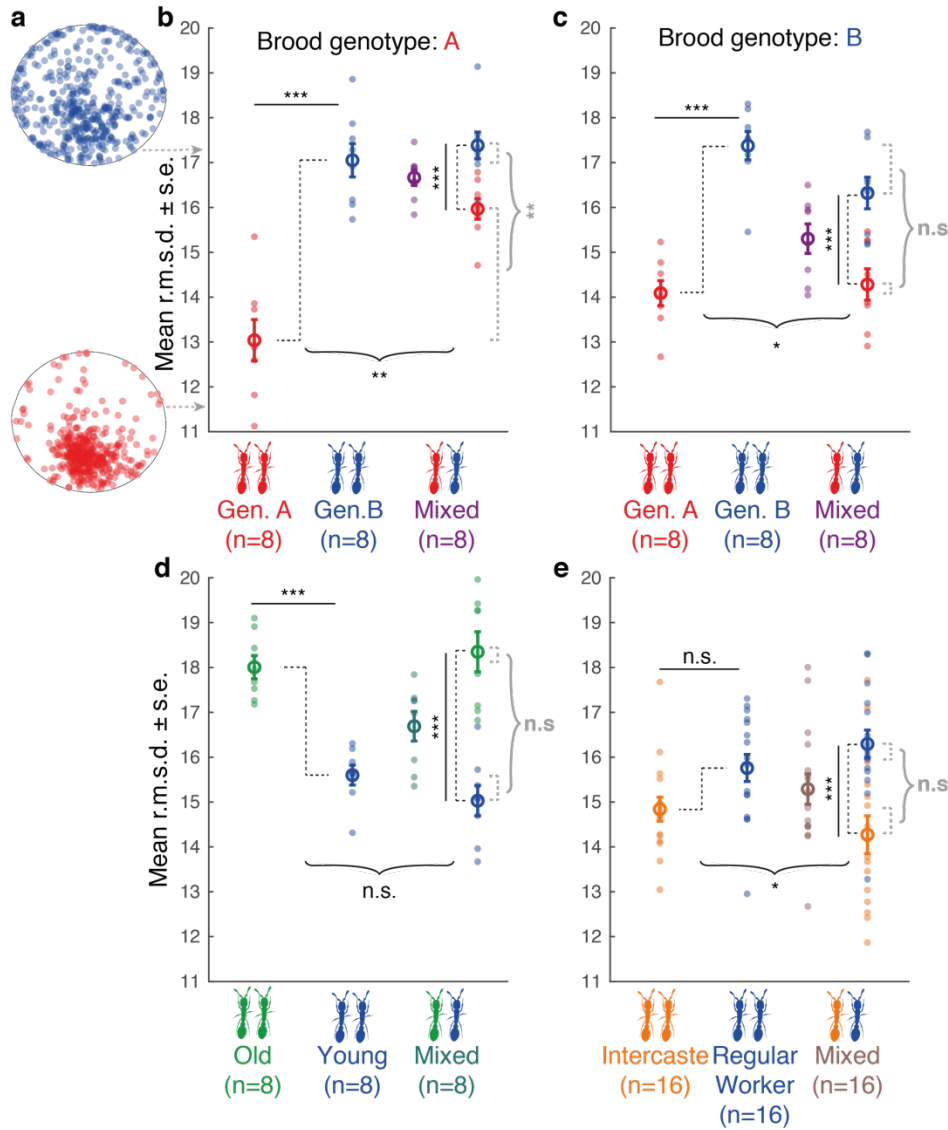
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790 **Figure 2. Mean r.m.s.d. (a proxy for mean extranidal activity) as a function of colony composition.**

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

24

802   brood. Colony size 16. GLMM post hoc Tukey tests (B_p vs. A_p: z = 7.75, p = $3.64*10^{-14}$; B_m vs. A_m : z =

803   4.61, p = $8.06*10^{-06}$) **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 = $2.80*10^{-13}$; B_m vs. A_m : z = 7.68, p = $6.57*10^{-14}$) **d:** Behavior as a

805   function of colony demographic composition. Colony size 16 ($Young_p$ vs. Old_p: z = -6.05, p = $4.39*10^{-09}$;

806    $Young_m$ vs. Old_m : z = -13.31, p < $2*10^{-16}$). **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 < $2*10^{-16}$). n.s.: non-significant, *: p < 0.05, **: p < 0.01, ***: p < 0.001.

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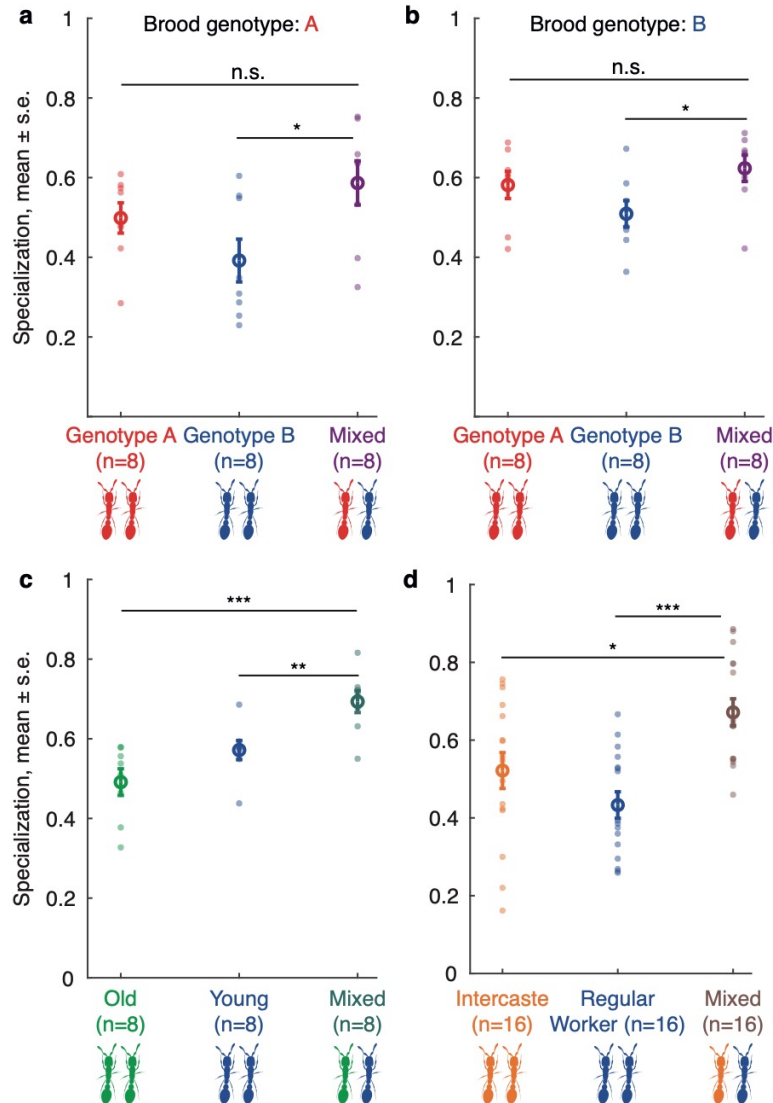
Figure 3. Specialization (day-to-day rank correlation in r.m.s.d.) as a function of colony composition. Opaque circles represent replicate colonies. Open circles represent average values across replicate colonies. Sample sizes indicate the number of replicate colonies. Identical colors across panels indicate ants of the same genotype, age, and morphological types. **a**: Specialization as a function of colony genetic composition in colonies with A brood. Colony size 16. (GLM post hoc Tukey tests; B_p vs. mixed: z = -2.78, p = 0.02; A_p vs. mixed: z = 1.25, p = 0.26) **b**: Specialization as a function of colony genetic composition in colonies with B brood. Colony size 16. (B_p vs. mixed: z = -2.41, p = 0.047 ; A_p vs. mixed: z = 0.88, p = 0.38) **c**: Specialization as a function of colony demographic composition. Colony size 16 (Young$_p$ vs. mixed: z = 3.01, p = 0.005; Old$_p$ vs. mixed: z = 5.01, p = $1.63*10^{-06}$) **d**: Specialization as a function of colony morphological composition. Colony size 8 (Regular Worker$_p$ vs. mixed: z = -4.35, p = $4.05*10^{-05}$, Intercaste$_p$ vs. mixed: z = 2.73, p = 0.013). n.s.: non-significant, *: p < 0.05, **: p < 0.01, ***: p < 0.001.

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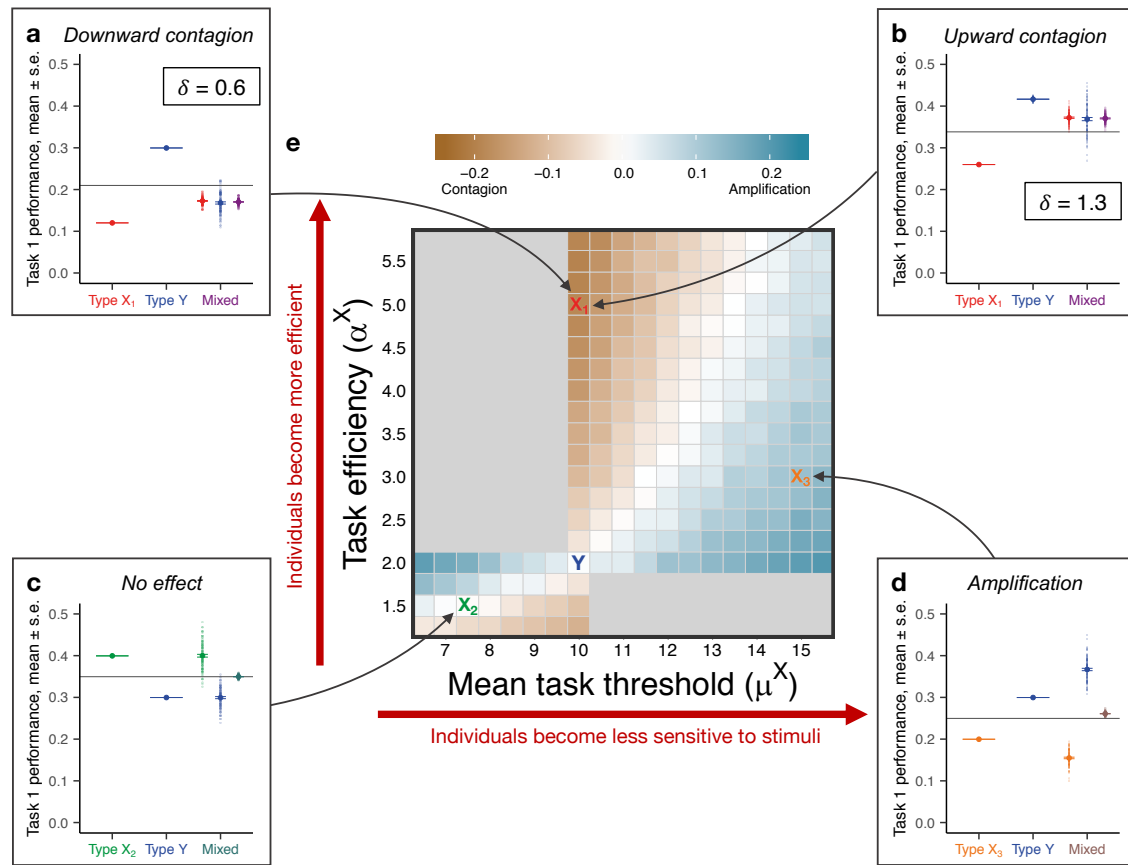
Figure 4. Theoretical predictions of the FTM on task performance and their robustness. a-d: Task performance frequency as a function of colony composition. One hundred replicates were simulated for each colony composition. Each opaque circle represents a replicate colony (colony size 16); each solid circle represents the average value across all replicates for its corresponding colony (or sub-colony) composition. Horizontal gray lines represent the average value of the pure colonies (first two columns) in their respective panels. Identical colors across panels indicate ants of the same types; in particular, the parameters for type Y ants are fixed across panels **a-d** ($\mu^Y = 10$, $\alpha^Y = 2$). **a-b:** Differences in task efficiency (α) between types and demand rate (δ) across colonies capture asymmetric behavioral contagion, downward (**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 more efficient than type Y ($\alpha^{X_1} = 5$, $\alpha^Y = 2$). Type Y can keep up with demand when the larvae are less demanding (**a**) but not when they are more demanding (**b**); type X_1 can keep up with the demand in both cases. Parameters: $\sigma = 0.1$, $\mu = 10$, $\eta = 7$, $\tau = 0.2$. **c-d:** Between-type differences in task efficiency (α) and mean threshold (μ) capture both a lack of effects from mixing (**c**) and behavioral amplification (**d**). In **c**, 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 ($\mu^{X_2} = 7.5$, $\mu^Y = 10$). 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 ($\mu^{X_3} = 15$, $\mu^Y = 10$). Parameters: $\sigma = 0.1$, $\eta = 7$, $\delta = 0.6$, $\tau = 0.2$. **e:** Change in between-type relative task 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    $\sigma = 0.1$, $\tau = 0.2$.
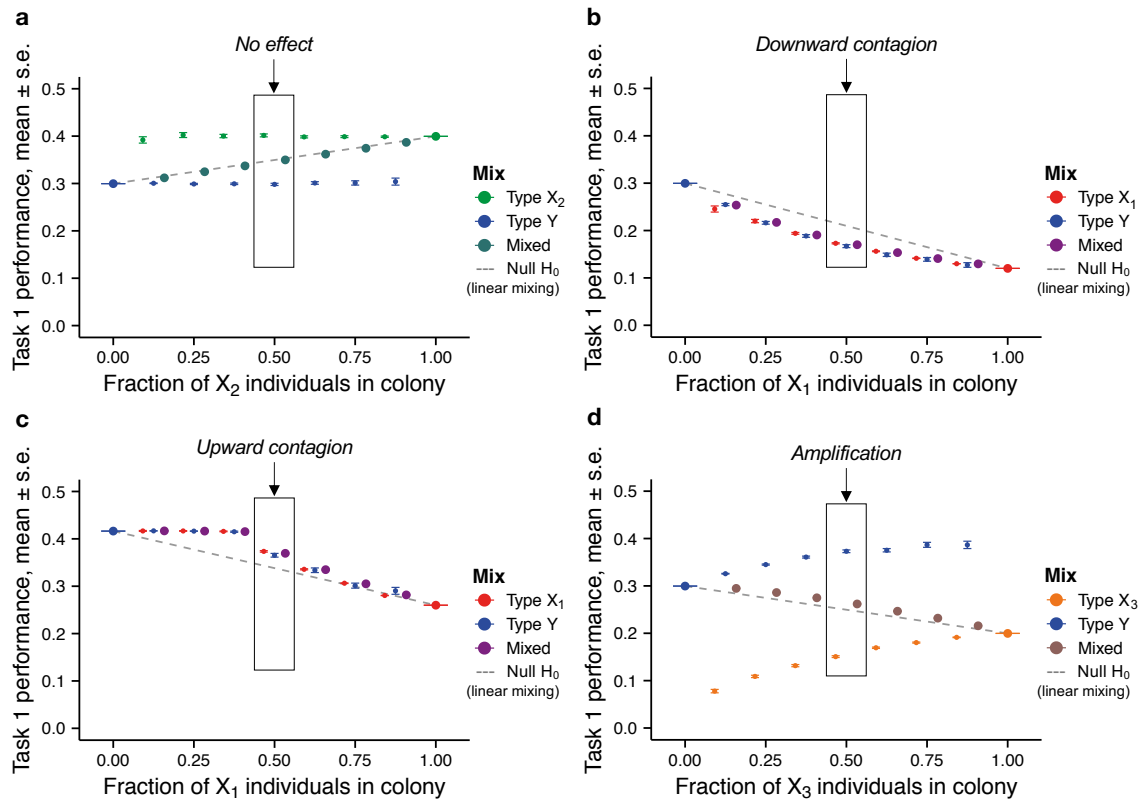
Figure 5. Predictions of the FTM for non-1:1 mixes. Colonies of size 16 with varying ratios of X and Y individuals were simulated under different conditions of threshold values, task-performance efficiency, and task demand. One hundred replicates were simulated for each colony composition. Each large circle represents the mean for that mix of X and Y individuals, while the neighboring smaller circles represent the means of X and Y individuals, respectively, within that mix. The dashed lines indicate the null hypothesis of linear behavioral effects of mixing types. The boxes highlight the behavioral patterns that characterize the 1:1-mixes, and their labels indicate correspondence with panels in Fig. 4 (**a** with Fig. 4c, **b** with Fig. 4a, **c** with Fig. 4b, and **d** with Fig. 4d). Parameters for each type (X_1, X_2, X_3, Y) are identical to those of the corresponding type in Fig. 4. **a**: X_2 individuals have a lower mean task threshold and are less efficient than Y individuals. **b**: X_1 individuals are more efficient than Y individuals. **c**: X_1 individuals are more efficient than Y individuals, but task demand is high. **d**: X_3 individuals have a much higher mean task threshold than 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)	*Identical* across colonies of different ant types (P1)	Exhibits DOL	*Identical* to pure colonies (P1)	Higher DOL than in pure colonies (P2)	Behavioral *amplification only* (P3)
	Extended FTM (with variation in other biologically relevant params)	*Different* across colonies of different ant types (P4)	Exhibits DOL	*Different* from pure colonies (P4)	Higher DOL than in pure colonies (P2)	Behavioral *contagion, amplification, or neither* (P5)

🟩 = experiments agree with prediction

🟥 = experiments disagree with prediction

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