Influence of the social context on division of labor in ant foundress associations

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13   Short title: Division of labor in ant foundress associations.

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17   ABSTRACT

18   Previous studies indicate that division of labor can arise spontaneously in social

19   groups. The comparison between normally social populations and forced associations

20   of solitary individuals allows us to dissect the mechanisms by which tasks are

21   distributed within a group and to ask how selection acts on division of labor during

22   the incipient stages of sociality. In some ant species newly mated queens form

23   cooperative associations during nest initiation, in which individuals specialize on

24   different tasks. The harvester ant Pogonomyrmex californicus shows geographical

25   variation across populations in colony founding strategies: solitary founding

26   (haplometrosis) and group founding (pleometrosis). This system provides a unique

27   opportunity to investigate how social context affects division of labor during social

28 evolution. We created groups containing either normally solitary, normally group

29 founding or mixed groups of solitary and social queens to examine how social

30 phenotype affects division of labor. We also examined how group size affects task

31 specialization by comparing pairs of queens with groups of six queens. Division of

32 labor arose consistently across all associations. Groups of haplometrotic or

33 pleometrotic queens differentiated into an excavation and a brood care specialist. In

34 mixed groups, the haplometrotic queens took the role of excavator while the

35 pleometrotic queens mainly tended brood. Our data also show that the intensity of

36 specialization was greater in larger associations, consistent with current models of

37 group size and division of labor. We discuss these data in the context of how

38 emergence and selection act on the evolution of division of labor within incipient

39 social groups.

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44   INTRODUCTION

45   Division of labor, where individuals within a group perform different roles, is

46   found recurrently across diverse social taxa, including shrimps (Duffy et al. 2002),

47   dung beetles (Hunt and Simmons 2002), caterpillars (Underwood and Shapiro 1999),

48   rats (Grasmuck and Desor 2002), lions (Stander 1992), dolphins (Gazda et al. 2005),

49   birds (Bednarz 1998) and extensively in insects (Wilson 1971; Hölldobler and Wilson

50   1991). High levels of division of labor also appear spontaneously in artificially

51   created social groups of normally solitary individuals in different species of ants

52   (Fewell and Page 1999; Helms Cahan and Fewell 2004) and solitary bees (Sakagami

53   and Maeta 1987; Jeanson et al. 2005). The comparison of social populations with

54   normally solitary ones allows us to dissect the mechanisms by which tasks are

55   distributed within a group, and to address the question of how selection acts on

56   division of labor during the early evolution of sociality.

57   In social insects, newly mated females initiate colonies either solitarily

58   (haplometrosis) or in cooperative groups (pleometrosis). Pleometrosis has been

59   reported across a variety of taxa including several species of termites (Roisin 1993),

60   halictine bees (Packer 1993), ants (Bernasconi and Strassman 1999), wasps (Itô 1987)

61   and thrips (Morris et al. 2002). These associations are often of unrelated individuals

62   (Kukuk and Sage 1994; Danforth et al. 1996; Bernasconi and Strassman 1999;Hacker

63   et al. 2005). Thus, although kin selection might shape cooperation in mature colonies,

64   individuals within pleometrotic associations cooperate and specialize on different

65   tasks during colony founding despite being unrelated. Because tasks vary in

66   physiological costs and risk, the costs of specialization in these associations are borne

67   individually, while the benefits are generally shared commonly (Rissing et al. 1989;

68   Bernasconi and Keller 1998; Helms Cahan and Fewell 2004). This variance may

69 actually lead to constraints on the levels of division of labor seen within cooperative

70 groups, because the maintenance of sociality may favor task sharing rather than task

71 specialization (Fewell and Page 1999; Helms Cahan and Fewell 2004).

72   In initial stages of colony founding, ant foundresses cooperate in nest

73 construction, and lay eggs in a common pile tended by all queens. Some species, such

74 as *Pogonomyrmex californicus*, also forage during the period before workers emerge,

75 a high risk task. Excavation can also increase predation risk and is especially costly

76 for desert ant species, because it can cause increased water loss via abrasion of the

77 cuticle (Johnson 2000). In foundress associations of *P. californicus* or *P. barbatus*, the

78 queen that becomes the excavator specialist has a correspondingly higher probability

79 of mortality. In contrast, brood tending may provide an overlooked nutritional benefit

80 because queens often consume a subset of the eggs (Helms Cahan 2001). Previous

81 work has shown that the degree of specialization for excavation is often higher in

82 forced associations of normally solitary foundresses than in pleometrotic groups

83 (Fewell and Page 1999; Helms Cahan and Fewell 2004), raising the question of

84 whether the evolution of sociality involves a shift in division of labor towards task

85 sharing for particularly costly (or beneficial) tasks.

86   In the seed-harvester ant *P. californicus*, populations display geographic

87 variation in their nest founding strategies, including both solitary and group founding

88 (Rissing et al. 2000). Two geographically close populations send out alates for mating

89 at similar times; we use this unique opportunity to combine normally solitary and

90 normally group founding queens into mixed associations, and compare their task

91 behavior to that of associations in which foundresses are either all haplometrotic or all

92 pleometrotic. While previous studies on *Pogonomyrmex* focused only on a unique

93 task (excavation: Fewell and Page 1999; Helms Cahan and Fewell 2004), we ask how

94   the presence of concurrent tasks, excavation and brood care, affect division of labor.

95   From the hypothesis that pleometrosis involves the evolution of task sharing to

96   minimize costs disparities, we predict haplometrotic associations to exhibit a higher

97   degree of division of labor than pleometrotic associations.

98   We also expect that the roles taken by queens within mixed associations will

99   differ, reflecting the different evolutionary histories of the two populations. In a study

100   of mixed groups of normally solitary and social *Messor pergandei*, Helms Cahan

101   (2001) found that the non-social individual invested more into colony growth (but not

102   brood care) and performed the costly task of excavation more frequently, with

103   consequently lower survival than the normally social queen in the pair. In the context

104   of division of labor, we expect that haplometrotic queens would be more likely

105   become the excavation specialist, the more costly task, because they have not been

106   selected to behave adaptively in social contexts in which cost disparities arise. In

107   contrast, pleometrotic queens in mixed groups should tend to reduce costs associated

108   with founding by reducing performance of costly tasks relative to their performance

109   by the other queen.

110   Nest surveys in the field report variation in the size of pleometrotic harvester

111   ant foundress associations from two to over twenty foundresses (Rissing et al. 2000).

112   Theoretical (Gautrais et al. 2002; Jeanson et al. 2007) and experimental studies

113   (Karsai and Wenzel 1998; Thomas and Elgar 2003) show that enhanced specialization

114   should parallel increased group size. Thus, we also examine how foundress group size

115   shapes patterns of task specialization by comparing foundress pairs with groups of six

116   queens. We expect larger associations to display greater division of labor, due to the

117   combination of a reduction in the need for work to be completed relative to the

118   number of available individuals (Jeanson et al. 2007).

119

120   METHODS

121   *Studied species*

122   Solitary (haplometrotic) and group founding (pleometrotic) queens were

123   collected on 3-6 July 2004 during the mating flight season (late June through mid-

124   July). Pleometrotic queens were collected 5 km north of Cameron Fire Station, San

125   Diego County, California. In this area, 75% of new nests contain multiple foundresses

126   (Rissing et al. 2000). Haplometrotic queens were collected 4 km north of Morretis

127   Junction, San Diego County, California. At this site, no evidence of nest co-founding

128   has been found (RA Johnson, personal communication). All collected queens were

129   newly mated; they had shed their wings and were walking on the ground, but had not

130   yet begun nest excavation. Queens were placed in closed containers with moistened

131   paper towels. Within each population, we ranked queens by weight, measured to the

132   nearest 0.1 mg, and divided the set in half. Queens were individually marked with

133   enamel paint on thorax and abdomen and associated with ants of the same weight

134   group. We paired either haplometrotic or pleometrotic queens, or one queen of each

135   population (mixed pairs); these treatments are referred to as association type. We also

136   formed associations of either six haplometrotic queens, six pleometrotic queens, or

137   three ants of each population (mixed groups).

138

139   *Experimental set-up*

140   Queens were introduced into horizontal observation nests (width: 12.5 cm,

141   length: 17.5 cm, inner thickness: 0.32 cm) filled with moist soil that had been

142   collected from the nesting area of pleometrotic queens and passed through a 2 mm

143   sieve. Each nest was connected with a plastic tube (length: 3 cm, inner diameter: 0.6

144 cm) to an arena (diameter: 8.5 cm, height: 4 cm). Nests were maintained in a walk-in

145 incubator at 35°C and a 12h:12h photoperiod. In total, we formed 22 haplometrotic

146 pairs, 16 pleometrotic pairs, 18 mixed pairs, 13 haplometrotic groups of 6 ants, 12

147 pleometrotic groups of 6 and 14 mixed groups of 6. Experiments were performed in

148 two successive replicates, with 52 and 43 nests observed in the first and second

149 replicate respectively. The composition of nests was balanced between replicates, and

150 replicates were pooled for analysis. At the end of each replicate, the excavated soil

151 was collected, dried and weighed. Excavated soil masses were compared with a two-

152 way ANOVA to test for the influence of group size and association type on amount

153 excavated.

154

155 *Behavioral observations*

156 Observations began immediately upon introduction of ants into the arena

157 connected to each nest. During surveys, we scanned each nest and recorded the

158 behaviors displayed by each queen (behaviors described below). The completion of a

159 session of five surveys required approximately two hours. We allowed a latency of

160 twenty minutes between sessions. In total, four sessions, with five surveys per session,

161 were performed daily over four consecutive days (20 observations per queen per day).

162 Kentucky blue grass seeds were provided *ad libitum* in the arena throughout the four

163 days. However, few bouts of foraging were observed during the time span of the

164 experiment and this task was not included in analyses.

165 We recorded the following behaviors: excavating (digging the soil or carrying

166 a pellet of soil in mandibles); brood tending (sitting on brood or holding/carrying the

167 egg mass within mandibles); inactive (sitting away from brood). Foundresses began

168 laying eggs within 24 hours after their introduction in the experimental set-up.

169 Agonistic interactions among queens (one queen biting another or carrying a living

170 nestmate away from nest) were also recorded. Whenever possible, we identified

171 which queen initiated agonistic interactions.

172

173 *Sample size and mortality*

174 Some queens died over the course of the experiment, but there was no bias in

175 mortality between haplometrotic and pleometrotic queens. When one queen died in a

176 pair, behavioral observations ended for that pair. In total, five haplometrotic pairs (out

177 of 22 pairs), four pleometrotic pairs (out of 16 pairs) and three mixed pairs (out of 18

178 pairs) were excluded from the analysis because of queen mortality. In the three mixed

179 pairs discarded, one haplometrotic and two pleometrotic queens died. For groups of 6

180 ants, we stopped observations if two or more ants died. Two haplometrotic groups of

181 6 queens (out of 13), one pleometrotic (out of 12 pairs) and four mixed groups (out of

182 14 pairs) were discarded because of mortality. In the four mixed groups excluded

183 because of mortality, a total of five pleometrotic and three haplometrotic foundresses

184 died. One group of 6 pleometrotic foundresses did not excavate and was discarded

185 from analysis. The data collected for nests of 5 or 6 ants were pooled in analyses to

186 preserve sample size. In total, 11 haplometrotic groups (including 6 nests with 5

187 queens), 10 pleometrotic (including 4 nests with 5 queens) and 10 mixed associations

188 (including 4 nests with 5 queens) were considered for analyses.

189

190 *Quantification of division of labor*

191 Two tasks were considered in analysis of division of labor (DOL): excavation

192 and brood care. Inactivity was not considered a task. The *DOL* statistic measures the

193 degree to which different individuals within a group specialize on different tasks and

194   the degree to which each individual is a specialist. To calculate *DOL*, we generated a

195   matrix of task performance, in which each cell contains the frequency with which a

196   specific individual was observed performing a specific task. The matrix was

197   normalized so that the total of all cells added to one. From this matrix we calculated

198   Shannon's index, $H_{(tasks)}$, for the distributions of individuals across tasks (see Gorelick

199   et al. 2004 for detailed methodologies) and mutual entropy for the entire matrix.

200   Mutual entropy divided by the Shannon's index $H_{(tasks)}$ yields an index that ranges

201   from 0 (no division of labor) to 1. We used two-factor repeated measures ANOVA on

202   values of DOL_i followed by post-hoc Tukey tests to test for the effects of day, group

203   size and association type on the intensity of division of labor.

204   We used Monte Carlo simulations resampling techniques to determine whether

205   the degree of differentiation in task performance arising in pairs was greater than

206   would be expected under random variation alone. Because individual task

207   performance was affected by group size (see below), the number of bouts

208   characterizing each individual in the simulations was randomly drawn from the

209   individual performance measured in associations and not from single foundresses. In

210   each iteration of the simulation, we formed groups of 2 or 6 queens with their

211   individual performance for excavation and brood care being randomly drawn with

212   replacement from the experimental distribution of the number of bouts performed

213   daily by individuals within experimental associations. For each group size (2 or 6

214   foundresses), we simulated haplometrotic (or pleometrotic) associations by sampling

215   the distribution of the number of bouts measured experimentally in pure associations

216   of haplometrotic (or pleometrotic) queens. The individual performance of

217   haplometrotic and pleometrotic ants in mixed simulated associations was randomly

218   drawn from the experimental distribution of the number of excavation and brood care

219 bouts obtained in pure associations of haplometrotic and pleometrotic queens

220 respectively. In total, we performed 1000 iterations for each condition (association

221 types and group size). The daily values of DOL_i between experimental and simulated

222 associations were compared with a repeated measures ANOVA.

223

224 *Behavioral differentiation within nests*

225 We examined how the composition of nests (group size and founding pattern)

226 affected task specialization. For each pure association of haplometrotic or

227 pleometrotic queens, we first ranked ants by their individual performance of

228 excavation; in pairs the individual that excavated more frequently was identified as

229 the Higher Frequency Excavator (HFE), and the queen excavating less frequently the

230 Lower Frequency Excavator (LFE). These designations were maintained for

231 comparisons of brood care, to determine whether the individual who performed more

232 excavation was also likely to perform more of the other task, whether pairs showed a

233 division of labor in which the individual who excavated less tended to brood more

234 frequently, or finally whether distribution of the two tasks across individuals was

235 random. For mixed groups, queens were clustered depending on their metrosis

236 (haplometrosis or pleometrosis) and then compared for their individual performance

237 of the two focal tasks. In pure associations of six queens, we identified three HFE and

238 three LFE to allow the comparison of their performance with the one achieved by the

239 haplometrotic or pleometrotic foundresses within mixed associations of six queens.

240

241 *Daily activity patterns and total number of behavioral bouts*

242 We assessed daily activity patterns within nests by summing the total number

243 of bouts spent inactive per day for each nest, divided by the number of queens and

244   multiplied by the number of scans. Data were compared with a two-factor repeated-

245   measures ANOVA after arcsine transformation. The total number of excavation and

246   brood care bouts performed daily per nest was compared after square root

247   transformation with a two-factor repeated-measures ANOVA to examine the

248   influence of group size and association type on overall work output.

249

250   All statistical tests were two-tailed and performed with SPSS v.12 for

251   Windows (SPSS Inc., Chicago, USA).

252

253   RESULTS

254

255   *Intensity of division of labor*

256   A two-factor ANOVA test with repeated measure across days was used to

257   determine effects of association type (haplometrotic, pleometrotic or mixed) and

258   group size (groups of two or six) on the daily values of *DOL*. There was no variation

259   over days in *DOL* (two-way repeated measures ANOVA: $F_{3,213}=1.0$, $P=0.39$) (Fig. 1).

260   Calculated *DOL* in groups of 6 queens was generally higher than in pairs ($F_{1,71}=9.99$,

261    $P=0.002$). Association type also significantly influenced *DOL* ($F_{2,71}=7.62$, $P=0.001$).

262   The intensity of division of labor was lower in haplometrotic than in either

263   pleometrotic (post-hoc Tukey test: $P=0.001$) or mixed associations (post-hoc Tukey

264   test: $P=0.004$), but there was no difference in *DOL* between pleometrotic and mixed

265   associations (post-hoc Tukey test: $P=0.89$). There was no interaction between group

266   size and association type on *DOL* ($F_{2,71}=2.02$, $P=0.14$) (Fig. 1). Across days, *DOL*

267   (mean ± SE) equalled 0.32 ± 0.07 in haplometrotic pairs, 0.50 ± 0.10 in pleometrotic

268   pairs and 0.56 ± 0.09 in mixed pairs (Fig. 1). In associations of 6 queens, mean *DOL*

269   was 0.51 ± 0.08 in haplometrotic groups, 0.71 ± 0.09 in pleometrotic groups and 0.57

270   ± 0.09 in mixed groups. The intensity of division of labor was significantly higher in

271   experiments than expected from random in Monte Carlo resampling in all types for

272   each of the treatment groups (repeated-measures ANOVA, 0.001<P<0.03) with the

273   exception of haplometrotic pairs (repeated-measures ANOVA: $F_{1,1013}$=0.05, P=0.82).

274

275   *Total number of behavioral bouts displayed by nest*

276   To examine how group size and nest type affected overall task performance,

277   we computed the total number of excavation and brood care bouts performed daily in

278   each nest (Fig. 2). Task performance changed significantly across the four days; the

279   number of excavation bouts decreased dramatically, while brood care bouts increased

280   (Figure 2; Table 1). There was a significant interaction between group size and days

281   for both tasks (Table 1). On day one, groups of six performed 1.22 times as many

282   excavation bouts as pairs (mean ± SE: 23.3 ± 2.1 vs. 19.1 ± 1.1). However, excavation

283   quickly decreased within the larger groups, so that on day four pairs actually

284   excavated more than groups of six (7.7 ± 0.9 vs. 3.8 ± 1). For brood care, groups of

285   six performed 5.8 times as many bouts as pairs on day one (13.4 ± 1.7 vs. 2.3 ± 0.5);

286   on day four, this decreased to a ratio of 3.4 (43.1 ± 1.7 vs. 12.5 ± 0.8). Throughout

287   this period the differences in excavation between groups of two versus six remained

288   consistently lower than differences in brood care. Overall brood care increased

289   significantly with group size but excavation did not (Fig. 2). Association type

290   marginally influenced brood care and excavation (Table 1). Haplometrotic

291   foundresses tended to perform more excavation bouts than pleometrotic queens and

292   mixed groups tended to engage more in brood care than pleometrotic associations (Fig.

293   2). The interaction between group size and association type on task performance was

294   not significant (Table 1).

295

296   *Behavioral differentiation within nests*

297   In haplometrotic pairs, the queen who excavated more frequently (HFE) on

298   average performed 66 ± 6% of excavation while her nestmate performed 63 ± 6% of

299   brood care bouts (G test: χ^2=7.10, *df*=1, *P*<0.01). In pleometrotic pairs, the HFE

300   queen performed 77 ± 6% of excavation and her nestmate 70 ± 6% of brood care

301   bouts (G test: χ^2=16.07, *df*=1, P<0.001). In all mixed pairs (n=15), the haplometrotic

302   queen performed more excavation bouts than the pleometrotic queen, averaging 85 ±

303   6 % of total excavation bouts (G test: χ^2=46.15, *df*=1, *P*<0.001). Conversely, the

304   pleometrotic queens performed more brood care than haplometrotic foundresses in all

305   but two mixed pairs, averaging 81 ± 5 % of total brood care bouts (Fig. 3a). This

306   pattern persisted with increasing group size. In mixed associations of 6 queens (n=15),

307   pleometrotic queens performed 69 ± 5% of brood care, while haplometrotic

308   foundresses performed 82 ± 3% of excavation bouts (G test: χ^2=24.84, *df*=1, *P*<0.001)

309   (Fig. 3b). In haplometrotic groups, the three queens who excavated more frequently

310   performed 84 ± 3% of excavation, while their nestmates did 55 ± 5% of brood care

311   bouts (G test: χ^2=23.76, *df*=1, *P*<0.001). In pleometrotic groups, three queens

312   performed 89 ± 2% of excavation while their nestmates achieved 64 ± 5% of brood

313   care bouts (G test: χ^2=37.06, *df*=1, *P*<0.001).

314

315   *Activity*

316   We computed the total proportion of time spent inactive by queens within each

317   nest. There was no variation in activity pattern across days (repeated-measures

318   ANOVA: $F_{3,213}=0.56$; $P=0.64$). The relative amount of time each queen spent inactive

319   increased with group size from 49 % for pairs to 65% for groups of six queens

320   (repeated-measures ANOVA: $F_{1,71}=30.76$; $P<0.001$). The different types of

321   associations behaved similarly in terms of activity patterns (repeated-measures

322   ANOVA: $F_{2,71}=2.75$; $P=0.07$) but the associations of pleometrotic foundresses tended

323   to be more inactive than mixed or haplometrotic associations.

324

325   *Agonistic interactions*

326   Over the four days of observations, no agonistic interactions (defined as a ant

327   biting another or carrying a living nestmate way from the nest) was observed in

328   pleometrotic associations. At least one aggressive interaction was observed in 23%

329   and 20% of haplometrotic and mixed pairs, respectively. Similarly, 27% of

330   haplometrotic and 60% mixed associations of 6 foundresses displayed some agonistic

331   interactions. A total of 12 haplometrotic queens were observed to initiate agonistic

332   interactions across all nests. The aggression was directed toward 8 haplometrotic and

333   8 pleometrotic queens. In another 11 incidents, 10 haplometrotic and 1 pleometrotic

334   queen were involved in aggression but the initiator and recipient could not be

335   distinguished. There was no difference in body mass of haplometrotic queens

336   initiating or receiving agonistic acts (*t*-test: $t_{18}=0.66$, $P=0.52$).

337   Within any association type, there was no difference in the intensity of

338   division of labor (DOL_i) as a function of the presence or absence of agonistic

339   interactions (*t*-test, haplometrotic pairs: $t_{15}=0.31$, $P=0.76$; mixed pairs: $t_{13}=0.11$,

340    $P=0.92$; groups of 6 haplometrotic queens: $t_9=1.23$, $P=0.25$; mixed groups of 6 queens:

341    $t_8=1.84$, $P=0.10$). (No tests were performed for pleometrotic associations because

342   agonistic interactions were absent).

343

344 *Excavation and soil removal*

345   In all nests, groups dug a single gallery. There was a significant influence of

346 association type on the mass of soil excavated (two-way ANOVA: $F_{2,71}=3.20$,

347 $P=0.047$). Haplometrotic associations excavated more soil (mean ± SD: 7.3 ± 2.6 g)

348 than pleometrotic associations (mean ± SD: 5.7 ± 1.8 g) (post hoc Tukey test: $P=0.04$);

349 there were no differences in amount excavated between haplometrotic and mixed

350 associations (mean ± SD: 6.8 ± 2.0 g) (post hoc Tukey test: $P=0.80$) or between

351 pleometrotic and mixed associations (post hoc Tukey test: $P=0.47$). Interestingly, the

352 mass of soil excavated did not differ between pairs (mean ± SD: 6.8 ± 2.4 g) versus

353 groups of six queens (mean ± SD: 6.3 ± 2.0 g) (two-way ANOVA: $F_{1,71}=0.92$,

354 $P=0.34$). There was additionally no interaction between association type and group

355 size on amount of soil excavated (two-way ANOVA: $F_{2,71}=1.15$, $P=0.32$). Regardless

356 of nest size or association type, queens dug about 5-10 % of the available nest volume

357 (soil mass within nest=111 grams).

358

359 DISCUSSION

360   We examined the emergence of task specialization in associations of ant

361 foundresses from two populations of *P. californicus* that differed in whether they

362 initiated nests alone or in groups. In our study, some individual task specialization and

363 consequent division of labor appeared in each of the foundress types: groups of

364 normally solitary-founding queens, group-founding queens and mixed groups of

365 foundresses from the two populations. Our data are mixed in terms of their fit to

366 predictions based on the hypothesis of division of labor as an emergent property. In

367 previous studies, we have found either equally high or higher levels of task

368   specialization or division of labor in forced associations of normally solitary

369   individuals. In this study, our measures of division of labor were lowest in the

370   normally solitary (haplometrotic) associations, and not significantly different from

371   random in haplometrotic pairs. However, we found the highest levels of division of

372   labor within mixed associations of haplometrotic and pleometrotic queens, consistent

373   with the hypothesis that task differentiation is generated in part from intrinsically

374   based variation in task propensity. Most associations from both the haplometrotic and

375   pleometrotic populations differentiated into excavation and brood care specialists.

376   However, when placed in mixed pairs, the haplometrotic queens consistently took the

377   role of excavator, a task with high physiological costs (Johnson 2000). Also consistent

378   with the emergence model, the level of *DOL* increased as group size varied from two

379   to five or six individuals (Jeanson et al 2007).

380   Haplometrosis, or solitary nest founding, is likely the ancestral condition for

381   harvester ants as for most ant species (Johnson 2004), but pleometrosis occurs in

382   several ant taxa in dry environments, including *Pogonomyrmex*, *Messor* (seed

383   harvesting ants, Rissing and Pollock 1986) and *Acromyrmex* (desert leaf-cutting ants:

384   Rissing et al. 1989, 1996) suggesting a benefit for the evolution of cooperative nest

385   construction in this ecological context. These associations generally show some level

386   of corresponding division of labor (Helms Cahan and Fewell 2004, Rissing and

387   Pollock 1986, Rissing et al. 1989, 1996). Although they eventually develop eusocial

388   colonies, until worker emergence these associations essentially function as quasisocial

389   groups of unrelated adults who participate cooperatively in nest construction and

390   brood rearing. Thus, we would expect that differential performance of different tasks,

391   and the costs and benefits for individuals within the group influence the evolution of

392   pleometrotic associations similarly in other cooperatively breeding social systems.

393   According to the response threshold model, division of labor should emerge

394   whenever members of a social group show some initial differentiation in their

395   propensities to perform different tasks (Bonabeau et al. 1998; Robinson and Page

396   1989). In *Pogonomyrmex*, the excavation specialist in a pair often can be predicted

397   from prior excavation performance while alone (Fewell and Page 1999; Helms Cahan

398   and Fewell 2004); the foundress with the higher propensity to dig while alone

399   generally assumes the role of specialist in the group. From these initial differences in

400   task preference, group dynamics can produce division of labor in associations of

401   normally solitary individuals (Fewell and Page 1999, Jeanson et al. 2005). According

402   to this model, division of labor should emerge even within associations of normally

403   solitary individuals; in previous experiments we found that indeed task specialization

404   and division of labor are as high or higher in groups formed from normally

405   haplometrotic populations compared to pleometrotic associations, including in *P.*

406   *californicus* (Helms Cahan and Fewell 2004).

407   Our current data correspondingly show differentiation of individual queens

408   into an excavation and a brood care specialist. However, levels of differentiation

409   within the haplometrotic population used in this study are lower than in previous

410   studies, although they still showed significant DOL when placed in groups of six, a

411   biologically relevant group size (in the field groups range to over 20 individuals,

412   Johnson 2004; pers. obs.). It is worth noting that this haplometrotic population is

413   geographically separate from other haplometrotic *P californicus* studied previously,

414   but close to the pleometrotic population in this study, so that these data do not

415   represent a reversal of behavioral findings for populations previously studied. One

416   possibility is that the presence of agonistic interactions at low levels may have

417   disrupted task performance to some degree. Although aggression was not generally

high, haplometrotic queens in this population did initiate aggressive behaviors more frequently than pleometrotic queens or queens from other haplometrotic *P californicus* populations (Helms Cahan and Fewell 2004). If aggression can disrupt division of labor, then the degree of tolerance within a social group could be an interesting influence on task organization.

Differentiation of group members into task roles within foundress associations is expected to be based on intrinsic differences in task propensities (Beshers and Fewell 2001; Helms Cahan and Fewell 2004), coupled with the expectation that individuals to adjust their behaviors to the tasks performed by conspecifics. In this case, the lower intensity of division of labor in associations of normally solitary foundresses may originate from a poorer feedback from activities performed by nestmates. This is partly supported by the fact that associations of haplometrotic foundresses dug larger nests than groups of pleometrotic queens (although this could also be a reflection of population differences in preferred nest size). However, the absence of difference in nest size between associations of two and six haplometrotic ants indicate that normally solitary foundresses in actuality to modulate their behaviors depending on the presence of nestmates.

It could be argued that division of labour was lower in haplometrotic associations because normally solitary foundresses were less flexible in their behavioural repertoire and have to initiate excavation before they can attend brood care, thus reducing the opportunity for specialisation. This hypothesis can be discarded because foundresses in all types of associations engaged in both tasks on the first day and no difference in the collective dynamics of task performance was evidenced between nest types across days (Fig. 2).

442   Models of the early evolution of social groups must factor in both the benefits

443   of division of labor in terms of group efficiency and productivity, and its potential

444   costs. Indeed, the tasks involved in nest construction, brood care and foraging do not

445   carry equal cost or risk. Excavation carries a particularly high cost for ant foundresses;

446   the cuticular abrasion that occurs as a queen carries soil through tunnels to the surface,

447   increases desiccation risk (Johnson 2000). Conversely, by sitting close to brood pile,

448   queens perform less energetically costly tasks and/or may consume eggs laid by

449   nestmates (Helms Cahan 2001).

450   A second prediction associated with the evolutionary transition from solitary

451   to cooperative groups is that individuals within cooperative groups (but not solitary

452   individuals by comparison) should have some mechanism for adjusting their behavior

453   to reduce individual costs relative to others in the group. In support of this prediction,

454   we found that task roles in mixed pairs were predictable based on the metrosis of

455   foundresses, so that normally haplometrotic queens became excavation specialists and

456   pleometrotic queens tended brood. Similarly, in mixed associations of *Messor*

457   *pergandei*, non-social foundresses performed about one and a half more excavation

458   bouts than social queens in mixed pairs, but suffered a greater mass loss and were

459   more likely to die than their pleometrotic nestmates (Helms Cahan 2001). If we make

460   the assumption that between-population variance in individual task propensity is

461   higher than within-population variance, then the higher intensity of task

462   differentiation within mixed groups supports the expectation that division of labor is

463   generated at least in part from intrinsic variation in individual task sensitivity or

464   response thresholds. Mixed pairs would be expected to be more genetically dissimilar

465   and correspondingly to show higher levels of task differentiation. Finally, the abrupt

466   geographical shift from haplometrotic to pleometrotic populations found by Cahan et

467 al (1998) for *M. pergandei* also suggests that colony founding strategy is associated

468 primarily with intrinsic differences associated with genotypic variation, rather than a

469 flexible response to local changes in environmental conditions (Cahan et al. 1998).

470   One of the goals of this study was to explore the structure of division of labor

471 within foundress associations differing in group size. Groups do not necessarily need

472 to increase output for all tasks linearly to benefit from larger size. For example, nest

473 excavation needs to be performed quickly (and larger groups excavated faster), but it

474 is not necessarily an advantage for groups of six to produce a nest three times as large

475 as would a group of two, especially if desiccation costs of construction are high. In

476 support of this, the volume excavated remained constant across our different groups.

477 We can speculate that the perception of a critical nest volume or the size of the brood

478 pile may represent a cue leading to the reduction of digging rate. This reduction in per

479 capita work output can in turn affect division of labor via its effect on work demand:

480 the need (or availability) of work relative to the availability of workers to perform it.

481 In a simulation model, Jeanson et al. (2007) proposed that increasing group size

482 increases specialization, in part because demand decreases. Demand goes down as

483 colony size increases, because the availability of the additional individuals to perform

484 tasks increases faster than the increased work imposed on the colony by their presence.

485 We should not expect equal changes in work output across all tasks, however, and

486 indeed we did not find the same result for brood care as for excavation. In our study,

487 the total number of brood care bouts performed by groups of 6 ants were about three

488 times larger than in pairs, irrespective of the social composition of foundresses

489 associations (Fig. 2).

490   The differentiation of individuals within groups into those with costly versus

491 beneficial roles may provide a barrier to the evolution of sociality by generating cost

492   disparities among group members. This then raises the question of how task

493   specialization and conversely task sharing, evolve within societies. For social systems

494   to persist evolutionarily in the face of task costs, selection may act to reduce the

495   expression of specialization via task sharing, and consequently reduce fitness

496   disparities associated with division of labor. Alternatively, any costly asymmetry

497   between queens can be maintained only if the consequent division of labor yields

498   strong enough benefits to outweigh the individual costs for the individual "losing" by

499   their association with the more costly task (Helms Cahan and Fewell 2004). This is

500   consistent with the assertion that pleometrosis in ant foundresses is a case of

501   multilevel selection, in which fitness benefits should be considered at both the

502   individual and group levels (Dugatkin 2002, Korb and Heinze 2004, Wilson 1990).

503   For ant foundresses, different ecological pressures influence pleometrosis. An

504   evolutionary response to brood raiding (Rissing and Pollock 1987, 1991), limited nest

505   sites (Tschinkel and Howard 1983) or reduction of exposure to predators and

506   desiccation (Pfennig 1995) have been invoked to account for cooperative founding.

507   Moreover, pleometrosis confers several advantages that might outweigh the initial

508   costs of group founding, including enhanced individual survival (Johnson 2004) and

509   colony growth (Tschinkel and Howard 1983; Sasaki et al. 2005), better defense

510   against predators (Adams and Tschinkel 1995; Jerome et al. 1998), improved nest

511   construction (Peeters and Andersen 1989) and larger production of a brood raiding

512   force (Rissing and Pollock 1991). Finally, one might hypothesize that specialization

513   may arise inevitably as a consequence of the absence of relatedness among

514   foundresses, facilitating the expression of the interindividual variability in response

515   thresholds, and consequently promoting the behavioral differentiation of foundresses

516   (Oldroyd and Fewell 2007).

517   In conclusion, our results indicated that the interindividual behavioral

518   variability coupled with the social context generated by their nestmates produce

519   emergent effects on division of labor. These factors and group size all influence the

520   intensity of task specialization in foundresses associations and its consequent effects

521   on work output and fitness. In particular, pleometrotic foundresses appear to be more

522   sensitive to the social context and better able to modulate their behaviors depending

523   on tasks performed by their nestmates. This is consistent with expectations that

524   behavioral transitions are necessary for the evolution of groups in which individual

525   and group fitness are enhanced by social cooperation.

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533

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649

650 FIGURE LEGENDS

651

652 Figure 1: Daily mean DOL_i ± SEM for haplometrotic, pleometrotic and mixed

653 associations of two and six queens.

654

655 Figure 2: Daily total number of excavation and brood care bouts per nest performed

656 by haplometrotic, pleometrotic and mixed associations of two and six foundresses.

657 Error bars are not represented for clarity.

658

659 Figure 3: Mean number of excavation and brood care bouts performed per capita by (a)

660 pairs and (b) groups of 6 queens. For each population, foundresses were ranked

661 depending on their excavation performance.

662

663

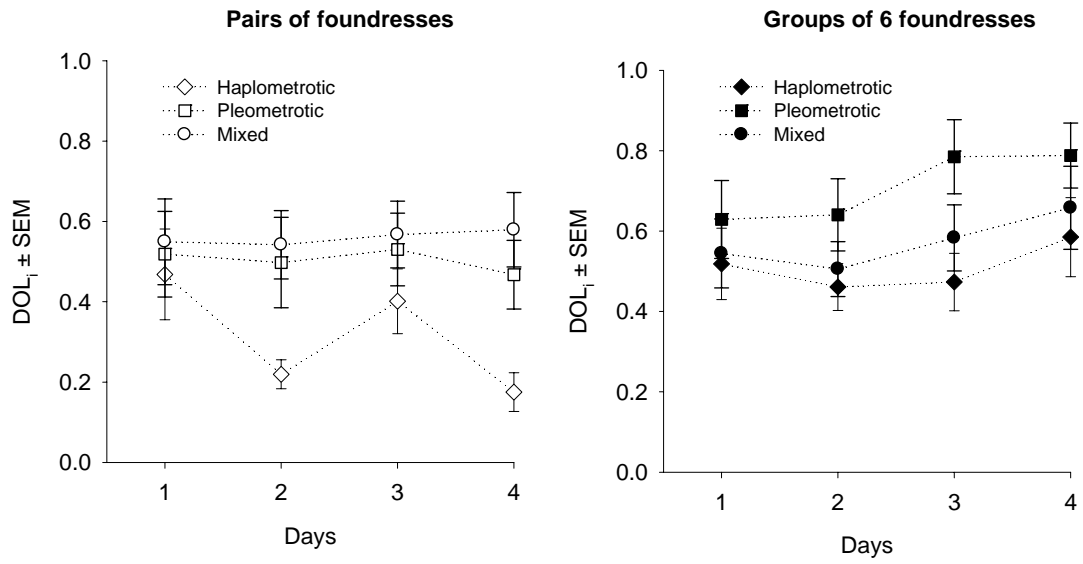| source of variation		d.f.	*F*	*P*
within subjects	task			
days	excavation	3	72.08	<0.001
	brood care	3	169.69	<0.001
days × association type	excavation	6	1.39	0.22
	brood care	6	0.2	0.97
days × group size	excavation	3	7.60	<0.001
	brood care	3	5.36	0.001
days × association type × group size	excavation	6	0.86	0.53
	brood care	6	0.45	0.85
between subjects				
association type	excavation	2	2.86	0.06
	brood care	2	3.07	0.05
group size	excavation	1	3.01	0.09
	brood care	1	355.24	<0.001
group size × association type	excavation	2	2.74	0.07
	brood care	2	0.29	0.75

Table 1: Summary of a two-way repeated measures ANOVA on the number of excavation and brood care bouts performed daily. Treatments were association type of foundresses (haplometrotic, pleometrotic and mixed) and group size (two or six foundresses).
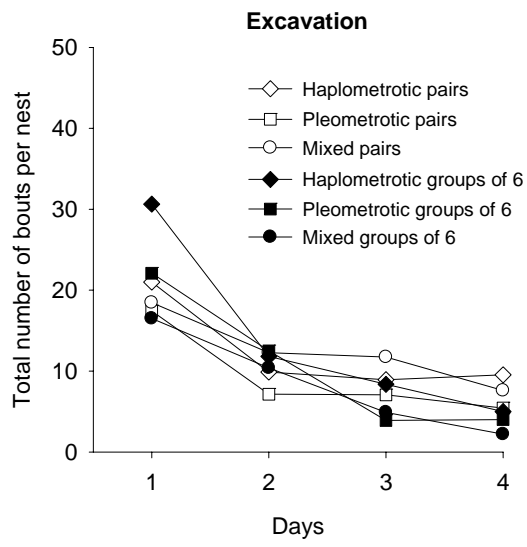
673   Figure 1

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675

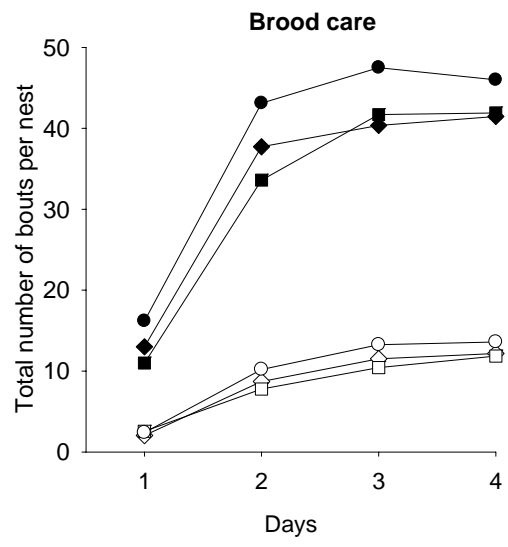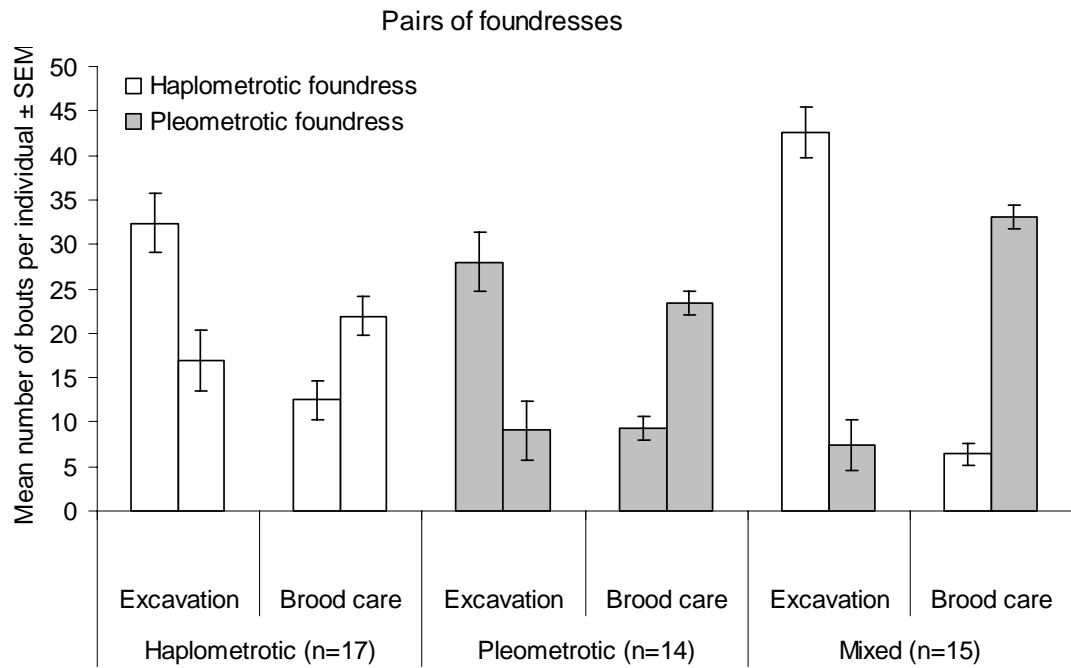**Pairs of foundresses**

DOL$_i$ ± SEM

◇ Haplometrotic
□ Pleometrotic
○ Mixed

Days

Groups of 6 foundresses

DOL$_i$ ± SEM

◆ Haplometrotic
■ Pleometrotic
● Mixed

Days

676

677   Figure 2

678

Excavation

Brood care

679   Days

680   Figure 3

681   a)

Pairs of foundresses

682

683   b)

Groups of 6 foundresses

684