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1 **Division of labour and socially-induced changes in response thresholds in associations of**
2 **solitary halictine bees**

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22 **ABSTRACT**

23 Division of labour is a recurrent property of social groups. Among the different
24 models proposed to explain the origin of division of labour, response threshold models have
25 garnered strong theoretical and empirical support. These models postulate that task
26 specialisation can arise spontaneously from inter-individual variation in thresholds for
27 responding to task-associated stimuli. Consequently, individuals with lower thresholds for a
28 given task are more likely to become specialists. Self-reinforcement models expand this
29 hypothesis by proposing that the successful performance of a task lowers an individual's
30 threshold, increasing the probability that it will perform that task again. Although an
31 important component of many models of division of labour, self-reinforcement can be
32 difficult to test in real-world contexts. Here, we asked whether social experience modulates
33 the individual response thresholds of normally solitary individuals. We focused on task
34 performance during the early stages of nest construction in forced associations of the
35 normally solitary halictine bee *Lasioglossum (Ctenonomia) NDA-1*. Within each pair, a strong
36 behavioural asymmetry arose with one bee specialising in excavation and her nestmate in
37 guarding the nest entrance. Individual performance of excavation by each bee was compared
38 before and after being paired with a conspecific. After experiencing a social environment,
39 individuals substantially increased their excavation performance. However, bees excavating
40 more frequently in groups did not excavate differentially more afterward, as would be
41 predicted by self reinforcement. The social context experienced by bees appears to promote
42 behavioural differentiation leading to task specialisation, and to modulate response thresholds
43 for excavation.

44

45

46 **Keywords:** division of labour, halictine bees, *Lasioglossum NDA-1*, nest construction,
47 response threshold, self-reinforcement, social modulation.

48 INTRODUCTION

49 Division of labour, where individuals within a group perform different roles, is a
50 recurrent property of sociality. Division of labour is believed to be a major determinant of the
51 ecological success of group-living species (Oster & Wilson 1978). It has been widely reported
52 in invertebrates, including shrimp (Duffy et al. 2002), caterpillars (Underwood & Shapiro
53 1999), thrips (Crespi 1992), hymenoptera (Wilson 1975, Oster & Wilson 1978, Hölldobler &
54 Wilson 1990), and termites (Gerber et al. 1988). Division of labour is also common in
55 vertebrates such as lions (Stander 1992), rats (Grasmuck & Desor 2002), dolphins (Gazda et
56 al. 2005), birds (Bednarz 1998), naked mole-rats (Sherman et al. 1991, Bennett & Faulkes
57 2000) and humans (Patterson et al. 2004).

58 Several models have been proposed to explain the origin of division of labour
59 (reviewed in Beshers & Fewell 2001). Of these, fixed threshold response models postulate
60 that division of labour can emerge spontaneously through variation among individuals in their
61 internal response thresholds to task-specific stimuli (Robinson and Page 1989; Bonabeau et
62 al. 1996, 1998). In performing a task, individuals with lower thresholds become the task
63 specialists and reduce the likelihood that the same task will be performed by individuals with
64 higher thresholds. Among eusocial insects, response thresholds have been empirically
65 demonstrated for several tasks including fanning (O'Donnell & Foster 2001, Jones et al.
66 2004), undertaking (Robinson & Page 1988) and foraging (Stuart & Page 1991, Fewell &
67 Page 2000). The requirements of this response threshold model are likely to be present at the
68 origins of sociality and may thus contribute to the emergence of division of labour in social
69 taxa. In support of this assertion, several studies have shown that division of labour can arise
70 spontaneously in groups of normally solitary ant foundresses (Fewell & Page 1999, Helms-
71 Cahan & Fewell 2004) and solitary bees (Sakagami & Maeta 1987, Jeanson et al. 2005).

72 In addition to fixed threshold models, some authors have proposed that experience can
73 modulate task-associated response thresholds via positive feedback (Deneubourg et al. 1987,
74 Plowright & Plowright 1988, Theraulaz et al. 1998). These self-reinforcement models
75 postulate that successful performance of a task can increase the probability of performing that
76 task again. Conversely, unsuccessful performance or lack of opportunity to perform the task
77 can reduce the likelihood that individuals will perform it, given later opportunities to do so.
78 Reinforcement can therefore produce worker specialization and influence the organisation of
79 work within colonies. Self-reinforcement effects on division of labour have some empirical
80 support in social insects. For instance, the individual response thresholds of bumble bee
81 workers for fanning to control nest climate vary with experience (Weidenmüller 2004).

82 Reinforcement can generate behavioural differentiation within an initially homogeneous
83 group of individuals. In the ant *Cerapachys biroi*, Ravary et al. (2007) found that division of
84 labour can arise spontaneously among originally undifferentiated individuals depending on
85 their foraging experience. Successful foragers became foraging specialists, while workers that
86 were unsuccessful in their initial foraging attempts became brood tending specialists.
87 Reinforcing mechanisms have also been proposed to contribute to the establishment of
88 dominance hierarchies, such as in wasps where hierarchical interactions reinforce the
89 probability that a given individual will dominate or loose in subsequent encounters (Theraulaz
90 et al. 1995, Camazine et al. 2001). However, tests of the contribution of self-reinforcement to
91 the production of division of labour are generally rare and are, to our knowledge, absent in
92 non-eusocial systems. This limits the application of the model to most social taxa. In addition,
93 when studied entirely within a social environment, the relative contribution of self-
94 reinforcement *per se* may be hindered by the influence of social interactions on division of
95 labour. An alternative approach is to test for persistent reinforcement effects on individual
96 response thresholds after task specialists and non-specialists have been separated.

97 Because their social systems range from solitary through communal, and in some
98 cases eusocial, halictine bees are a useful taxon to examine the evolution of division of
99 labour. In facultatively social bees, females may nest either solitarily or in groups depending
100 partly on ecological pressures, such as predation and nest availability (Dunn and Richard
101 2003). Division of labour between foraging and guarding frequently arises in these
102 associations. In carpenter bees of the tribe Xylocopini, nests established by solitary
103 foundresses may be later usurped by a second foundress with the dominant females
104 specialising in egg-laying and foraging while the non-reproductive females guard the nest
105 entrance (Hogendoorn & Velthuis 1999). Conversely, in ceratinine and allodapine bees,
106 dominant females usually guard the nests and subordinates forage (Hogendoorn & Schwarz
107 1998).

108 During the early stages of nest construction within forced pairs of the normally
109 solitary halictine bee *Lasioglossum (Ctenonomia) NDA-1*, previous work revealed that inter-
110 individual variation in the propensity to excavate, coupled with spatial separation generated
111 by social interactions, promotes the emergence of division of labour between tunnel
112 excavation and nest guarding (Jeanson et al. 2005). In the present study we ask whether social
113 experience can temporally modulate the individual response thresholds of normally solitary
114 bees. We test the prediction of self reinforcement that individuals increase their probability of
115 performing a task after becoming the specialist for that task. Focusing on the early stages of

116 nest construction in associations of the normally solitary bee *L. (Ctenonomia) NDA-1*, we
117 compare individual excavation performance before and after bees experience a social
118 environment. We also examine these artificially constructed social groups for the emergence
119 of division of labour.

120

121 **MATERIAL AND METHODS**

122

123 **Study species**

124 Females of the normally solitary *Lasioglossum (Ctenonomia) NDA-1* were collected in the
125 Cobboboonee State Forest (latitude: 141.53 E, longitude: 38.19 S, elevation: 70 m),
126 Southwestern Victoria, Australia during December 2006. A previous excavation of *L.*
127 (*Ctenonomia) NDA-1* revealed that only two of 19 nests contained two females, confirming
128 their status as primarily solitary nesting (McConnell-Garner and Kukuk 1997). Bees were
129 collected by netting above nest aggregations and were taken immediately to the laboratory,
130 where they were placed individually in Petri dishes supplied with a damp tissue and a 1:1
131 honey water mixture. The day before the first experiment, two groups of bees were marked
132 with enamel paint on the thorax and both sides of the abdomen. Bees were randomly assigned
133 to two treatments: a 'social' treatment where bees experienced the presence of a conspecific
134 during a portion of the experiment, and a solitary treatment (hereafter control bees) where
135 bees did not encounter conspecifics for the duration of the experiment. Bees were stored
136 overnight at ambient temperature (16°C) before experimental treatment.

137

138 **Experimental design**

139 We first quantified individual activity levels of each bee in the absence of nest construction or
140 social interactions, by measuring their movement rates in circle tubes (as per Breed et al
141 1978). We then placed bees individually into observation nests to assess their excavation
142 performance while alone. Following this, to assess the impact of social environment on task
143 performance, bees were separated into a control group of individuals that remained solitary,
144 and an experimental group in which bees were paired with others that showed initially similar
145 levels of excavation behaviour. All bees, solitary or paired, were placed into fresh observation
146 nests and observed for 18 hours. Following this, all bees were placed individually into new
147 nests and observed again to assess whether excavation behaviour was affected differentially
148 depending on social context (Figure 1).

149

150 *Activity level (Day 1)*

151 To examine whether differences in the nature of social interactions or task performance may
152 be influenced by differences in an individual's general activity level, we placed all bees
153 individually into a circular plastic tube (inner diameter: 0.42 cm, length: 20 cm), and
154 quantified the amount of time they were active (Fig. 1). The circular tube was placed in a
155 plastic box with long tubular lights on each side to ensure homogeneous lighting during data
156 collection. The temperature was maintained at 27 ± 0.5 °C. Each trial began 2 min after
157 introducing the bee into the box, and lasted for 5 min. During trials we recorded the
158 percentage of time a bee was walking or sitting/grooming and measured the time that
159 individuals took to cover a distance of 10 cm without stopping or turning. This experiment
160 took place between 09h30 and 13h00.

161

162 *Tunnel construction by single individuals (Days 1- 2)*

163 After assessment of activity levels, all bees were introduced individually (between 14h00 and
164 15h00) into observation nests (15 cm deep \times 12 wide cm \times 3 mm thick) and allowed to
165 excavate tunnels. Observation nests were filled with dampened sifted soil collected from the
166 nesting area in the field. Each nest was connected by a clear plastic tube (4 cm) to a clear
167 plastic vial (2-cm diameter, 4 cm high) containing a 1:1 honey water mixture. A small
168 indentation of 1 ± 0.1 cm was made in the soil to encourage the bee to dig a single vertical
169 gallery. Prior to introduction of bees, nests were cooled to 5 °C. After the bees were
170 introduced into the nests, the nests were placed in a climate controlled room at 15h00. The
171 climate controlled room was kept at 31 ± 1 °C with a photoperiod of 15 hours of light and 9
172 hours of darkness; darkness started at 19h00. The bees were allowed to excavate for 18 hours.

173 At 9h00 on day 2, the bees were removed from the observation nest and placed back in
174 their Petri dishes. Using a ruler, we measured the length of each bee's tunnel to quantify their
175 digging performance and assign them to treatment groups. We also digitally photographed
176 each nest to more precisely quantify tunnel depths using ImageJ (see below).

177

178 *Social interactions (Day 2)*

179 Bees were randomly assigned to either the solitary (n = 30 bees) or social treatment
180 groups (n = 35 pairs). Bees assigned to the social treatment were paired using individual
181 digging performance data. To minimize initial inter-individual differences in propensity to
182 dig, we matched individuals with similar digging performance (the two bees that dug the most
183 were paired, and so on, until the two bees that dug the least were paired). Prior to placing

184 them together in a nest, each pair was introduced into a circular plastic tube, under the same
185 light and temperature conditions described for activity levels, to quantify their social
186 interactions (see below) (Fig. 1). Observations began 2 min after bees were introduced, and
187 lasted 7 min. Three types of inter-individual encounters were observed in the social assays. In
188 all cases, the inter-individual distance of the two bees was less than 2 mm apart when these
189 encounters were recorded:

- 190 (1) Head to head encounter (a frontal encounter)
- 191 (2) Head to back encounter (a forward-moving bee encountered the back of a sitting bee)
- 192 (3) Back to back encounter (one or both bees backed in the tube and encountered each
193 other)

194 Once contact was established, we then recorded the outcomes of interactions. These
195 included:

- 196 (1) U-turn to head to head (following a head to back encounter, one bee made a U-turn
197 resulting in a head to head encounter)
- 198 (2) Withdraw (one bee made a U-turn and moved away from the other)
- 199 (3) Back (one bee backed away after encountering the other bee)
- 200 (4) C-posture (a bee curled her abdomen, giving a C-shaped position with the sting
201 pointed at the other female)
- 202 (5) Bite (one bee bit the other)
- 203 (6) Lunge (one bee quickly lunged toward the other)
- 204 (7) Block (one bee curled the abdomen to prevent the other bee from passing)
- 205 (8) Pass (bees passed by one another, requiring the rotation of each bee while moving past
206 the other venter to venter; we identified the bee initiating the pass, the bee allowing the
207 pass and instances where both bees mutually passed).
- 208 (9) No response (sitting and/or self-grooming)

209

210 Control bees were introduced individually into the circular tube under the same light and
211 temperature conditions for 9 min, but because they were alone, their social behaviour was not
212 assessed. This experiment took place between 10h00 and 12h30 on day 2.

213

214 *Tunnel construction by single and paired individuals (Days 2- 3)*

215 Following the circle tube assays for social interactions, we placed each solitary
216 treatment bee and each pair from the social treatment into different observation nests. Bees
217 were maintained in the same pairs for circle tube assays and observation nests. Four sessions

218 of twenty scans each were performed every 6 hr over the next 18 hr (**240** scans in total).
219 Individual nests were scanned every 5 min. During each scan we recorded the location and
220 behaviours of each bee, following Jeanson et al (2005). Behaviours consisted of:

- 221 (1) Excavate (the bee excavated dirt with her mandibles),
- 222 (2) Push (the bee pushed loose soil in the tunnel towards the nest entrance or vial),
- 223 (3) Tamp (the bee tamped loose dirt into side walls with her abdomen (Batra 1964)),
- 224 (4) Gate-Keep (the bee sits in the tube connecting the nest and the vial)

225 We also recorded all instances of sitting, grooming, walking and feeding from the
226 honey water solution in the vial. These behaviours were either not associated with specific
227 tasks or were rarely performed (feeding), and thus were not used in division of labour or task
228 specialization analyses. The tasks of pushing and tamping generally occurred together, and so
229 were combined for analyses.

230 Bee locations were categorized as: Vial, Tube, Tunnel, Bottom (dead end of a gallery).
231 The three focal tasks were spatially segregated within the nest; excavation occurred at the
232 bottom of a gallery, pushing/tamping took place in the tunnel or in the tube, and gate keeping
233 occurred close to the nest entrance. Excavation could only be performed by one bee at a time
234 within a tunnel, but pushing/tamping and gate-keeping could be performed by both bees
235 simultaneously. After 18 hours, the nests were digitally photographed to measure the lengths
236 of the tunnels.

237

238 *Tunnel construction by single individuals (Days 3- 4)*

239 To determine whether individual response thresholds changed with experience, bees
240 were removed from their paired (social treatment) or solitary (control treatment) nests and
241 transferred individually into new observation nests at 15h00 on day 3. After 18 hr, bees were
242 removed and the total tunnel length of each nest was measured. At the end of the experiments,
243 bees were euthanized and preserved in 95% ethanol.

244

245 **Data analysis**

246 *Tunnel length*

247 Each nest was photographed with a digital camera. From the pictures, the tunnels were
248 traced using the software ImageJ (<http://rsb.info.nih.gov/ij>) to precisely measure their total
249 length. There was a strong correlation between the total length and area of the tunnels
250 (Pearson correlation test: $r_{86}=0.92$, $P<0.001$). Because there was no variation in the width of
251 tunnels (which equalled approximately the width of a bee), we subsequently compared

252 excavation performance across treatments with reference to tunnel length. For the subsequent
253 analysis, we subtracted 0.9 cm to the length measured to take into account the indentation
254 made in the observation nests.

255

256 *Number of excavation bouts*

257 To quantify the influence of experience and social context on excavation behaviour,
258 we compared the number of excavation bouts performed across days. Because we did not
259 observe behaviour on days 1-2 or days 3-4, we used the relationship between excavation
260 bouts and tunnel length for control bees that dug alone on days 2-3 to estimate the number of
261 excavation bouts for all bees that dug alone on days 1-2 and days 3-4. There was a strong
262 positive correlation between tunnel length and the number of excavating bouts observed
263 (linear regression: $F_{1,29}=106.82$, $P<0.001$, $R=0.89$). The digging rate, estimated using the
264 inverse of the regression coefficient was 2.5 bouts/cm (i.e. the number of observed bouts
265 required to dig 1 cm; note this is the number of observed bouts, not the number of actual
266 bouts required to dig 1 cm, as each nest was only scanned 80 times during the 18 hour
267 period). Each bee's number of excavation bouts was then estimated for days 1-2 and days 3-4
268 by multiplying her tunnel length by the average digging rate.

269

270 *Quantification of division of labour*

271 Following Jeanson et al. (2005), we categorized individuals as performing one of three
272 tasks: excavating, pushing/tamping, or gate keeping. Division of labour within each pair was
273 quantified using an index developed by Gorelick et al. (2004). To calculate the index, we
274 generated a matrix of task performance, in which each cell showed the frequency with which
275 a specific individual was observed performing a specific task. The matrix was then
276 normalized so that the total of all cells added to one. From this matrix, we calculated the
277 Shannon's index $H(indiv)$ for the distribution of tasks across individuals (Gorelick et al.
278 2004). Dividing the mutual entropy between tasks and individuals by the marginal entropy of
279 individuals $H(indiv)$ gave the index $DOL_{tasks \rightarrow indiv}$. This index details how tasks are
280 distributed across individuals and ranges between 0 (no division of labour) and 1 (complete
281 division of labour). Pairs in which nestmates performed only one of the focal tasks or in
282 which one individual did not engage in any task were discarded.

283

284 *Statistical analysis*

285 The daily intensity of division of labour was compared using repeated measures
286 ANOVA. We used a MANOVA after square root transformation to compare the number of
287 behavioural bouts performed by pairs and control bees on days 2-3. We examined the
288 excavation performance over days between control bees and individuals within pairs by fitting
289 linear mixed-effects models (lme procedure, Pinheiro and Bates 2000) after square root
290 transformation of the number of excavation bouts. We started with a full model including time
291 (days), group (control, HFE, LFE; see below) and their first and second term interactions as
292 fixed effects. Observation nests and days were set as random factors. From the full model, the
293 minimal model was obtained by successively deleting non significant terms using the
294 parsimony principle based on the Akaike's Information Criterion (Pinheiro and Bates 2000).
295 The number of behavioural bouts performed individually on days 2-3 was compared using *t*-
296 tests after square root transformation. Pearson correlation tests were used to test the
297 relationship between tunnel length across successive days, the relationship between the
298 probability of passing and the intensity of division of labour, and the relationship between
299 activity level and excavation performance. Statistical tests were two-tailed and performed
300 with SPSS (v. 11.0, SPSS Inc., Chicago, USA), except linear mixed models which were done
301 using the statistical package R (R Development Core Team, 2005).

302

303 **RESULTS**

304

305 **Task performance in social versus solitary contexts**

306 We compared performance rates of solitary and paired bees for the three tasks: excavation,
307 pushing/tamping and gate keeping on days 2-3. Bees in the social treatments collectively
308 performed more behavioural bouts than control bees (Fig. 2). Pairs performed approximately
309 twice the number of excavations (MANOVA: $F_{1,62}=15.89$, $P<0.001$) and push/tamp bouts
310 (MANOVA: $F_{1,62}=28.50$, $P<0.001$) as control bees. Pairs also displayed about seven times
311 more gate-keeping than control bees (MANOVA: $F_{1,62}=106.44$, $P<0.001$). The total per
312 capita task performance was about 1.6 higher for pairs than for control bees (*t*-test: $t_{96}=4.62$,
313 $P<0.001$) and paired bees performed individually about 3.5 times more gate-keeping than
314 control bees (*t*-test: $t_{96}=4.96$, $P<0.001$).

315

316 **Differentiation within pairs for excavation behaviour**

317 Within each pair, we identified a higher frequency excavator (hereafter HFE) as the
318 bee performing more excavation bouts than her nestmate (hereafter LFE: lower frequency

319 excavator). One pair was discarded from this analysis because neither bee dug. In two pairs,
320 because both bees displayed the same number of excavation bouts, one bee was randomly
321 assigned as the HFE. Note that the first bee to be introduced into the nest was not more likely
322 to become the HFE (Chi² test: $\chi_1=0.13$, $P=0.72$).

323 We used linear mixed models to examine the variations in excavation performance
324 between HFE, LFE and control bees over time (Table 1). There was no difference in the
325 number of excavation bouts performed by control, HFE and LFE bees on Days 1-2 (Fig. 3).
326 There was a marginally significant variation in the excavation rates of control bees across the
327 three days of the experiment. When bees were paired together on days 2-3, their behaviour
328 diverged, such that one bee (HFE) excavated more than the other and LFE bees maintained a
329 similar excavation performance as on days 1-2. Pairs were separated on day 3 and individual
330 excavation behaviour was re-assessed in isolation. When placed alone in observation nests on
331 days 3-4, LFE bees dug more than they had while alone on days 1-2 or in pairs on days 2-3. In
332 contrast, HFE excavated more on days 2-3 and on days 3-4 than on days 1-2 but their
333 performance did not differ between days 2-3 and days 3-4. The tunnels dug by HFE and LFE
334 on days 3-4 were, respectively, 1.65 and 2.5 times longer than on days 1-2.

335

336 **Task specialization and level of division of labour in pairs**

337 Bees designated as HFE and LFE for excavation behaviour showed differential
338 performance of other tasks while together (G-test: $G_2=10.56$, $P<0.01$). There were no
339 differences between HFE and control (t -test: $t_{62}=1.9$, $P=0.06$), LFE and control (t -test:
340 $t_{62}=0.06$, $P=0.54$) or LFE and HFE in their pushing/tamping behaviour (paired t -test: $t_{33}=1.22$,
341 $P=0.23$) (for all tests, corrected $\alpha=0.017$) (Figure 4). Both LFE (t -test: $t_{62}=6.33$, $P<0.001$) and
342 HFE (t -test: $t_{62}=3.12$, $P=0.003$) displayed significantly more gate keeping bouts than did
343 control bees. The LFE bees also displayed significantly more gate keeping bouts than HFE
344 (paired t -test: $t_{33}=3.03$, $P=0.005$) (for all tests, corrected $\alpha=0.017$).

345 For each pair, the DOL metric was computed for each session of twenty scans during
346 the 18 hour period on days 2-3. One pair did not dig during days 2-3 and was excluded from
347 the analysis. For the three focal tasks, the total performance of each pair equalled at least 36
348 bouts (median=84; 1st quartile=67; 3rd quartile=101) and each bee within a pair performed at
349 least 11 bouts (median=54; 1st quartile=31; 3rd quartile=54). Division of labour did not change
350 over time (repeated-measures ANOVA: $F_{3,93}=0.27$, $P=0.85$); therefore data were pooled for
351 analysis. Across the four sessions of twenty scans (80 scans per pair), the average $DOL_{tasks \rightarrow}$
352 $_{indiv}$ equalled 0.26 ± 0.04 ($n=34$; median=0.13; 1st quartile=0.09; 3rd quartile=0.45). There was

353 no correlation between tunnel length and $DOL_{tasks \rightarrow indiv}$ (Pearson correlation test: $r_{34}=-0.28$,
354 $P=0.10$).

355

356 **Activity levels**

357 Control and paired bees did not differ in the amount of time they spent walking when they
358 were alone in the circle tubes (control mean \pm SEM: 0.37 ± 0.06 ; paired bees mean \pm SEM:
359 0.34 ± 0.03 ; t -test: $t_{98}=-0.47$, $P=0.64$). For paired bees, there was no correlation between
360 individual activity level (time spent walking) and the length of their tunnel dug alone on days
361 1-2 or on days 3-4 (Pearson correlations: days 1-2: $r_{70}=0.09$, $P=0.44$; days 3-4: $r_{70}=-0.18$,
362 $P=0.14$). Likewise, there was no correlation between activity level and tunnel length on any
363 of the three days for control bees (Pearson correlations days 1-2: $r_{30}=0.07$, $P=0.71$; days 2-3:
364 $r_{30}=0.33$, $P=0.07$, or days 3-4: $r_{30}=0.16$, $P=0.39$). Activity level did not predict which bees
365 would subsequently be categorized as HFE or LFE (HFE mean \pm SEM: 0.32 ± 0.05 ; LFE
366 mean \pm SEM: 0.39 ± 0.05 ; t -test: $t_{66}=-1.18$, $P=0.24$).

367

368 **Social interactions**

369 The median number of encounters observed per pair of bees was 11 (1st quartile=6.5; 3rd
370 quartile=18.5). Fourteen of 35 pairs displayed at least one C-posture (a total of 15 bees). In all
371 but one of these pairs only one bee displayed C-postures. The bees that displayed C-postures
372 did not differ from their counterparts in activity level (C-posture bee mean \pm SEM: $0.33 \pm$
373 0.06 ; other bee mean \pm SEM: 0.34 ± 0.04 ; t -test: $t_{68}=-0.2$, $P=0.84$). Among the 15 bees
374 displaying C postures, half (7) later became the HFE. Twenty five of 35 pairs displayed at
375 least one pass (median=7; 1st quartile=2; 3rd quartile=11). Across pairs, the median proportion
376 of encounters ending in a pass was 0.28 (1st quartile=0; 3rd quartile=0.55). Whenever possible,
377 we identified which individuals initiated the pass during each encounter. There was no
378 difference in activity level between bees initiating more passes (mean \pm SEM: 0.32 ± 0.07)
379 and bees allowing passes (mean \pm SEM: 0.43 ± 0.05) (t -test: $t_{48}=-1.23$, $P=0.22$). In 22 out of
380 32 pairs with an identifiable HFE, it was possible to identify which bee initiated passes.
381 Among the bees that initiated more passes, 11 became HFE and 11 were LFE. There was no
382 correlation between the probability to pass in circle tubes and $DOL_{tasks \rightarrow indiv}$ (Pearson
383 correlation test: $r_{34}=0.10$, $P=0.58$).

384

385 **DISCUSSION**

386 In this study, we first examined whether division of labour can arise during the early
387 stages of nest construction in forced associations of solitary individuals. In our experimental
388 conditions, three tasks were available: excavation, gate-keeping and pushing/tamping. A
389 behavioural differentiation regularly occurred within pairs, with one bee specialising in
390 excavation at the bottom of the gallery and her nestmate primarily guarding the nest entrance.
391 Pushing/tamping, which is a spatially intermediate task, was performed equally by both
392 individuals. These results support our conclusions from earlier work that division of labour
393 can emerge in pairs of normally solitary halictine bees (Jeanson et al. 2005).

394 In the present study, bees were paired so to minimize the initial interindividual
395 differences in their propensity to dig. However, once in pairs, the bees altered their
396 behaviours. One bee specialised in excavation and performed about two times more
397 excavation bouts than when she was previously alone. In contrast, her nestmate decreased her
398 excavation performance while simultaneously increasing her gate keeping. These data support
399 the assertion that division of labour can spontaneously emerge from social dynamics, even at
400 the origins of sociality. The differentiation between bees that were initially similar in
401 behaviour before they were placed in social groups indicates that the observed task
402 specialization is indeed an effect of social dynamics and not simply a reflection of individual
403 variation within the group (Fewell & Page 1999). Similar patterns of task specialisation have
404 been found in associations of normally solitary foundresses of the harvester ant
405 *Pogonomyrmex californicus* (Helms Cahan & Fewell 2004, Jeanson & Fewell subm.).

406

407 **Self-reinforcement and task differentiation**

408 Under the hypothesis of self-reinforcement, the more an individual performs a task,
409 the lower its associated threshold becomes (Theraulaz et al. 1998). The excavation specialist
410 (HFE) was thus expected to subsequently display a higher propensity to dig after taking over
411 the task of excavation in pair groups. In our experiments, the HFE indeed performed more
412 excavation on days 3-4 than on days 1-2, after they dug intensively on days 2-3. However,
413 LFE performed the task even more frequently after experiencing the social context. Indeed,
414 the excavation performance of LFE was similar on days 1-2 and days 2-3 but dramatically
415 increased (by a factor 2.5) on days 3-4 and reached about 85% of the performance achieved
416 by the HFE specialists on days 2-3. Conversely, bees maintained alone did not vary in their
417 excavation performance across days. While self-reinforcement might account for the
418 increased performance of excavating specialists, it is unlikely to explain the enhanced level of
419 digging among non-specialist bees. The increased excavation behaviour of both specialists

420 and non specialists compared to their excavation behaviour prior to being paired suggests that
421 the earlier social context experienced by bees induced a delayed modulation of response
422 thresholds for excavation which cannot be explained by self-reinforcement alone, or any other
423 existing models of task performance and/or division of labour. In addition, division of labour
424 did not increase during the first 18 hours of nest construction, contrary to the hypothesis that
425 self-reinforcement amplifies behavioural differentiation over time.

426 Although a growing body of empirical evidence supports for the role of response
427 threshold variation in the production and maintenance of division of labour, few empirical
428 studies have examined the contribution of self-reinforcement to task specialisation. To our
429 knowledge, such evidence has been reported only in eusocial taxa, including ants (Ravary et
430 al. 2007), wasps (Theraulaz et al. 1995) and bumblebees (Weidenmuller 2004). Our finding
431 that self-reinforcement is absent during the early stages of nest construction in forced
432 associations of normally solitary individuals suggests that self-reinforcement may be a
433 derived organizational mechanism that enhances division of labour in evolved societies but
434 does not appear spontaneously in incipient groups. Alternatively, self-reinforcement may not
435 apply to all available tasks. Using a similar experimental paradigm, further studies should test
436 whether self-reinforcement is involved during nest construction in communal and eusocial
437 halictine bees that burrow in soil.

438

439 **Social modulation of excavation behaviour**

440 Although our data do not support self-reinforcement as the primary mechanism
441 driving task differentiation, they clearly show that excavation is socially modulated; both bees
442 showed a reduction in their individual response thresholds for excavation after they
443 experienced the presence of a nestmate. A different pattern has been documented in
444 associations of normally social individuals. For instance, the ant *Camponotus japonicus* dug
445 significantly larger amounts of sand in associations than when the same ants worked
446 separately but did not alter individual digging performance before and after being paired
447 (Chen 1937 a, b). Similarly, in social halictine bees such as the communal *L. (Chilalictus)*
448 *hemichalceum* (Jeanson et al. 2005) and the eusocial *L. zephyrum* (Michener et al. 1971),
449 individuals that experience repeated contact show enhanced digging activity over bees that are
450 housed without social contact. An increase in the propensity to express a behaviour when
451 others express it is known as social facilitation (Clayton 1978, Webster & Fiorito 2001).
452 Social facilitation could be attributed to a reduction in the individual response thresholds
453 associated with a specific task depending on the perception of conspecifics already engaged in

454 the same activity. A comparative study between populations with different social structures
455 should aid in our understanding of how social context-induced modifications in individual
456 response thresholds may have paralleled social transitions in halictine bees. In particular, we
457 predict that communal bees maintained alone should have higher individual response
458 thresholds for excavation than solitary bees because repeated social contact appears to be
459 mutually stimulating within social populations.

460 How did social modulation of excavation behaviour occur in our study? One
461 hypothesis is that exposure to a larger nest volume than usually experienced by single bees
462 stimulated paired individuals to reach similar depths after they were separated. Indeed, the
463 nests excavated by pairs were about two times larger than nests dug concurrently by solitary
464 control bees, and single individuals from the social treatment excavated longer tunnels the
465 next day. A direct test for this effect would involve placing individual bees in different-sized
466 tunnels and measuring their excavation performance when subsequently alone. At the
467 molecular level, we speculate that social modulation of task performance may have been
468 mediated by biogenic amines. In invertebrates, biogenic amines (e.g. octopamine, serotonin,
469 dopamine) are important modulators of behaviour (Pflüger and Stevenson 2005). Earlier
470 social experience has been shown to alter individual behaviours via changes in amine
471 concentrations in various species of arthropods including spiders (Punzo & Punzo 2001),
472 crustaceans (Huber et al. 2001), crickets (Stevenson et al. 2005) and ants (Cuvillier-Hot &
473 Lenoir 2006). In addition, changes in amine titres are a significant source of variation in
474 response thresholds and contribute to the regulation of division of labour in insect colonies.
475 For instance, amines modulate the response threshold of honeybee for sucrose (Scheiner et al.
476 2002), and octopamine enhances the foraging response to brood pheromones (Barron et al.
477 2002, Barron & Robinson 2005). In our study, we hypothesize that the social context
478 experienced by normally solitary bees affected their levels of amines, which may in turn have
479 induced a modulation of excavation behaviours. Further studies should investigate whether
480 modifications of individual response thresholds correlate with variation in amine titres
481 depending on social context.

482

483 **Aggression and task allocation**

484 Aggression and dominance interactions can contribute to the establishment of
485 reproductive division of labour both in invertebrates (e.g. ants: Monnin & Peeters 1999;
486 wasps: Sledge et al. 2001) and in vertebrates (e.g. spotted hyenas: Creel et al. 1997).
487 Aggressive interactions among eusocial insect workers can also regulate allocation for non-

488 reproductive tasks. In the wasp *Polybia occidentalis*, biting interactions among workers
489 modulate the probability of foraging (O'Donnell 2003, 2006). In the present study, the
490 initiator or recipient of aggressive acts (e.g. C-posture) was not more likely to specialise in
491 excavation or guarding within pairs, and division of labour apparently did not emerge through
492 social contention. However, this does not mean that social interactions did not contribute to
493 task specialisation. Indeed, Jeanson et al (2005) found that the low probability of passing in *L.*
494 *NDA-1* generated a spatial segregation of solitary bees within the nest, which consequently
495 reduced the turnover among tasks and indirectly reinforced task asymmetry with pairs
496 (Jeanson et al. 2005).

497

498 **Guarding in solitary and social contexts**

499 A surprising result of our study was the extreme increase in gate keeping behaviour by
500 the paired bees in comparison to when they were solitary. As with excavation, this task also
501 showed extensive task specialization, with the bee who excavated less frequently being more
502 likely to gate-keep. In our experiments, guarding was observed more frequently in pairs than
503 in solitary nests, and pairs dug nests that were twice as deep. On average, the entrance of the
504 nest was guarded 50% of the time by at least one bee in pairs versus less than 10% in solitary
505 nests. In pairs, only one bee excavated at a time and the time spent digging or sitting by the
506 HFE at the bottom of the tunnel equalled approximately the time spent by her nestmate sitting
507 in the tube. The increased performance in gate-keeping in pairs may have resulted from the
508 predominant occupancy of the bottom of the gallery by the excavation specialist while, as a
509 result, her nestmate mainly occupied upper part of the nest and engaged in gate-keeping.

510 Although there was no possibility of predators or other intruders in our experiment,
511 gate-keeping is behaviourally similar to guarding in a natural context. In many halictine
512 species, the nest consists of a burrow that enters the soil with lateral cells that are provisioned
513 with pollen and nectar (Michener 1964). The entrance of the nest is generally constricted and
514 fits the head or abdomen size of a bee. Bees guard the nest by blocking the nest entrance with
515 their head or by presenting their abdomen (Batra 1964, Knerer 1969). In the halictine bee
516 *Agapostemon virescens*, guarding is responsible for the accrued resistance to attack by
517 cleptoparasites in communal nests in comparison to solitary nesting (Abrams and Eickwort
518 1981). Abrams and Eickwort (1981) proposed that social nesting permits bees to (1) guard
519 nests at all times, (2) conserve digging effort per nestmate, and (3) dig deeper burrows
520 reaching soil levels with optimal water content. During the incipient stages of sociality,
521 associations of initially solitary individuals may therefore have benefited from improved nest

522 defence through increased guarding and improved ability to reach soils with optimal water
523 content. These benefits may have promoted transitions to communal sociality in halictine
524 bees.

525

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531

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689
 690 Table 1: Results of the linear mixed model fitted by maximum likelihood for the square root
 691 of the number of excavation bouts performed individually; with parameter estimates (i.e.
 692 fixed-effects estimates), approximate standard errors, degrees of freedom, t-estimates (ratio
 693 between the estimates and the standard errors) and P values. Control represents bees placed
 694 individually into observation nests. HFE and LFE represent bees within pairs; each bee within
 695 a pair was designated HFE (Higher Frequency Excavator) or LFE (Lower Frequency
 696 Excavator) based on the number of observed times they excavated while in the pair. Nests
 697 refer to observation nests. Formulae follow the convention of the R language.

698

699 *Random effects*

700 Formula: ~days|nests

701 Standard Deviation: Intercept=1.37 Days=0.54 Residual=1.35

702

703 *Fixed effects*

704 Formula: $\sqrt{(\text{individual excavation bouts})} \sim \text{group} \times \text{days} + \text{group} \times \text{days}^2$

705

	Estimate	SE	df	t-value	P
Intercept	2.07	0.17	222	11.86	<0.0001
Control × Days	1.18	0.56	222	2.08	0.04
HFE × Days	2.54	0.52	222	4.84	<0.0001
LFE × Days	-1.51	0.53	222	-2.87	0.004
Control × Days ²	-0.52	0.29	222	-1.79	0.07
HFE × Days ²	-0.98	0.27	222	-3.63	0.0004
LFE × Days ²	1.12	0.27	222	4.14	<0.0001

706

707

708 **Figure legends**

709

710 Figure 1: Sequence of experimental design.

711

712 Figure 2: Box plots of the total number of excavation, push/tamp and gate keeping bouts
713 performed by control bees and pairs on days 2-3. * indicates statistical significance. Boxes
714 show median value, 25th and 75th percentiles and outliers.

715

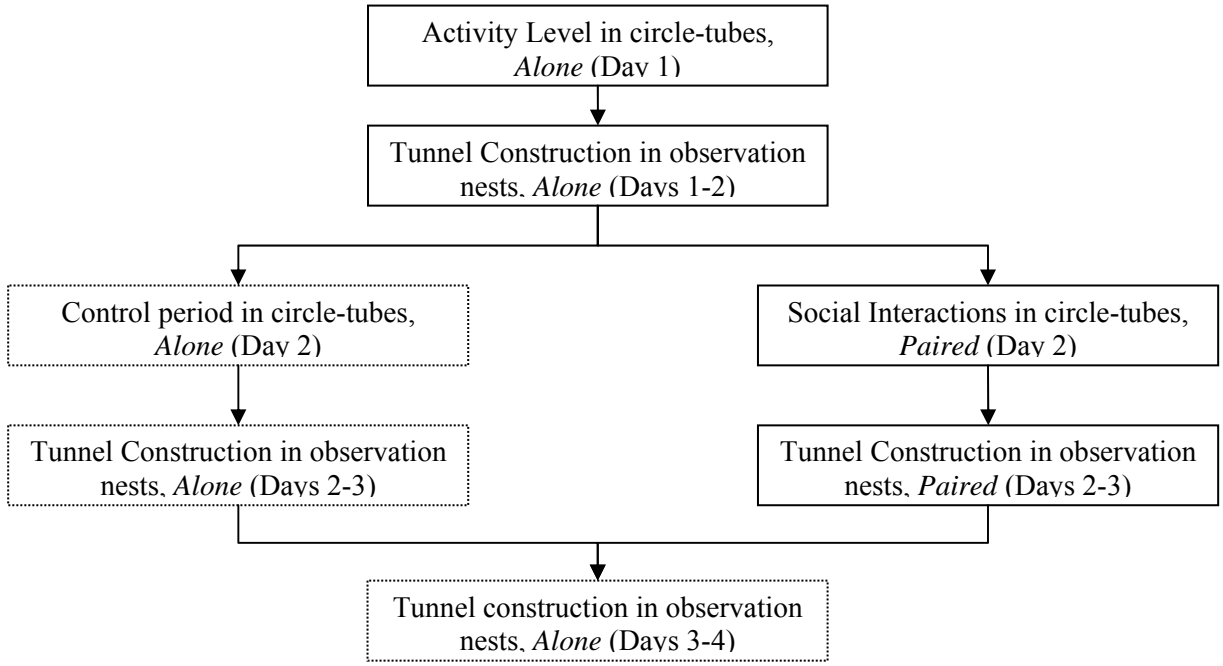
716 Figure 3: Box plots of excavation bouts performed daily by control, HFE (higher frequency
717 excavator, i.e. bee performing more excavation bouts than her nestmate within pairs), and
718 LFE (lower frequency excavator) bees. Boxes show median value, 25th and 75th percentiles
719 and outliers.

720

721 Figure 4: Box plots of the number of behavioural bouts performed by control, HFE (higher
722 frequency excavator) and LFE (lower frequency excavator) bees on days 2-3. For each
723 behaviour, different letters indicate significant statistical differences. Boxes show median
724 value, 25th and 75th percentiles and outliers.

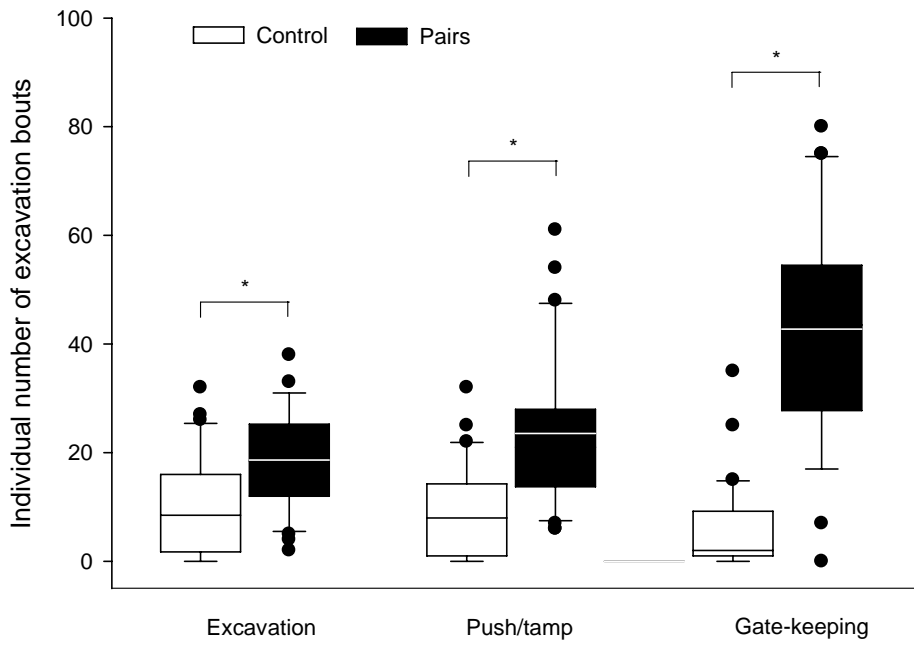
725

726 Figure 1
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728



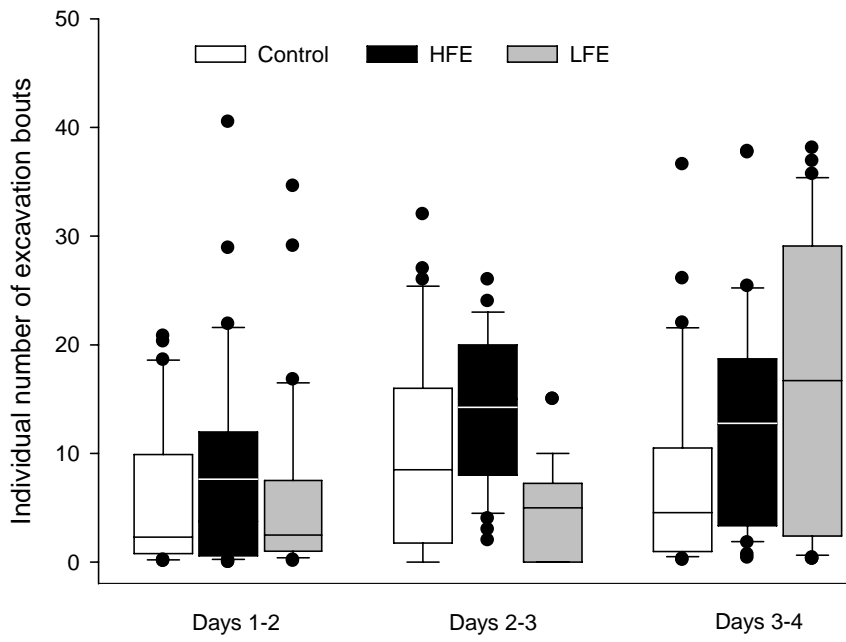
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730 Figure 2
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733

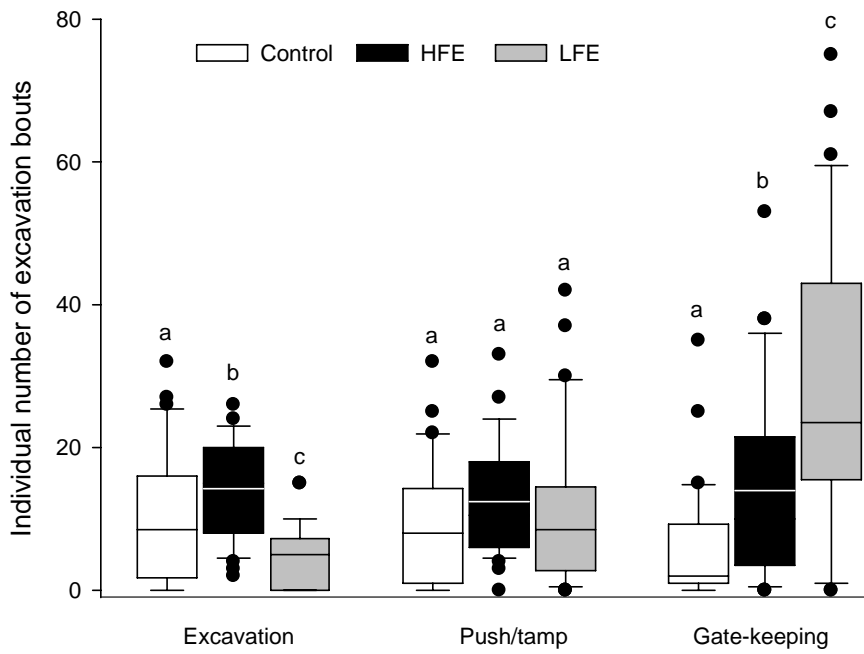
734 Figure 3
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736



737
738

739 Figure 4

740



741
742