



Division of labour and socially induced changes in response thresholds in associations of solitary halictine bees

RAPHAËL JEANSON*, REBECCA M. CLARK†, C. TATE HOLBROOK†, SUSAN M. BERTRAM‡, JENNIFER H. FEWELL† & PENELOPE F. KUKUK§

*Centre de Recherches sur la Cognition Animale, CNRS UMR 5169—Université Paul Sabatier, Toulouse

†School of Life Sciences, Arizona State University

‡Department of Biology, Carleton University

§Division of Biological Sciences, The University of Montana

(Received 8 November 2007; initial acceptance 18 December 2007;

final acceptance 26 March 2008; published online 3 June 2008; MS. number: 9577R)

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

© 2008 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Keywords: division of labour; halictine bee; *Lasiglossum NDA-1*; nest construction; response threshold; self-reinforcement; social modulation

Division of labour, in which individuals within a group perform different roles, is a recurrent property of sociality.

Correspondence: R. Jeanson, Centre de Recherches sur la Cognition Animale, CNRS UMR 5169—Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse, France (email: jeanson@cict.fr). R. M. Clarke, C. T. Holbrook and J. H. Fewell are at the School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, U.S.A. S. M. Bertram is at the Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada. P. F. Kukuk is at the Division of Biological Sciences, The University of Montana, Missoula, MT 59812-4824, U.S.A.

Division of labour is believed to be a major determinant of the ecological success of group-living species (Oster & Wilson 1978). It has been widely reported in invertebrates, including shrimp (Duffy et al. 2002), caterpillars (Underwood & Shapiro 1999), thrips (Crespi 1992), hymenoptera (Wilson 1975; Oster & Wilson 1978; Hölldobler & Wilson 1990) and termites (Gerber et al. 1988). Division of labour is also common in vertebrates such as lions (Stander 1992), rats (Grasmuck & Desor 2002), dolphins (Gazda et al. 2005), birds (Bednarz 1988), naked mole-rats (Sherman et al. 1991; Bennett & Faulkes 2000) and humans (Patterson et al. 2004).

Several models have been proposed to explain the origin of division of labour (reviewed in Beshers & Fewell 2001). Of these, fixed-threshold-response models postulate that division of labour can emerge spontaneously through variation among individuals in their internal response thresholds to task-specific stimuli (Robinson & Page 1989; Bonabeau et al. 1996, 1998). In performing a task, individuals with lower thresholds become the task specialists and reduce the likelihood that the same task will be performed by individuals with higher thresholds. Among eusocial insects, response thresholds have been empirically shown for several tasks, including fanning (O'Donnell & Foster 2001; Jones et al. 2004), undertaking (Robinson & Page 1988) and foraging (Stuart & Page 1991; Fewell & Page 2000). The requirements of this response-threshold model are likely to be present at the origins of sociality and may thus contribute to the early emergence of division of labour in social taxa. In support of this assertion, several studies have shown that division of labour can arise spontaneously in groups of normally solitary ant foundresses (Fewell & Page 1999; Helms Cahan & Fewell 2004; Jeanson & Fewell 2008) and solitary bees (Sakagami & Maeta 1987; Jeanson et al. 2005).

In addition to fixed-threshold models, some authors have proposed that experience can modulate task-associated response thresholds via positive feedback (Deneubourg et al. 1987; Plowright & Plowright 1988; Theraulaz et al. 1998). These self-reinforcement models postulate that successful performance of a task can increase the probability of performing that task again. Conversely, unsuccessful performance or lack of opportunity to perform the task can reduce the likelihood that an individual will perform it, given later opportunities to do so. Reinforcement can therefore produce worker specialization and influence the organization of work within colonies. Self-reinforcement effects on division of labour have some empirical support in social insects. For instance, the individual response thresholds of bumblebee workers for fanning to control nest climate vary with experience (Weidenmüller 2004). Reinforcement can generate behavioural differentiation within an initially homogeneous group of individuals. In the ant *Cerapachys biroi*, Ravary et al. (2007) found that division of labour can arise spontaneously among originally undifferentiated individuals depending on their foraging experience. Successful foragers became foraging specialists, whereas workers that were unsuccessful in their initial foraging attempts became brood-tending specialists. Reinforcing mechanisms have also been proposed to contribute to the establishment of dominance hierarchies, such as in wasps, among which hierarchical interactions reinforce the probability that a given individual will dominate or lose in subsequent encounters (Theraulaz et al. 1995; Camazine et al. 2001). However, tests of the contribution of self-reinforcement to the production of division of labour are generally rare and are, to our knowledge, absent in non-eusocial systems. This limits the application of the model to most social taxa. In addition, when studied entirely within a social environment, the relative contribution of self-reinforcement per se may be hindered by the influence of social interactions on division of labour. An alternative

approach is to test for persistent reinforcement effects on individual response thresholds after task specialists and nonspecialists have been separated.

Because their social systems range from solitary through communal, and in some cases eusocial, halictine bees are a useful taxon to examine the evolution of division of labour. In facultatively social bees, females may nest either solitarily or in groups depending partly on ecological pressures, such as predation and nest availability (Dunn & Richards 2003). Division of labour between foraging and guarding frequently arises in these associations. In carpenter bees of the tribe Xylocopini, nests established by solitary foundresses may be later usurped by a second foundress, with the dominant female specializing in egg laying and foraging while the nonreproductive female guards the nest entrance (Hogendoorn & Velthuis 1999). Conversely, in ceratine and allodapine bees, dominant females usually guard the nest and subordinates forage (Hogendoorn & Schwarz 1998).

Previous work revealed that, during the early stages of nest construction by forced pairs of the normally solitary halictine bee *Lasiglossum (Ctenonomia) NDA-1*, interindividual variation in the propensity to excavate, coupled with spatial separation generated by social interactions, promotes the emergence of a division of labour between tunnel excavation and nest guarding (Jeanson et al. 2005). In the present study we asked whether social experience can temporally modulate the individual response thresholds of normally solitary bees. We tested the prediction of self-reinforcement that individuals increase their probability of performing a task after becoming the specialist for that task. Focusing on the early stages of nest construction in associations of the normally solitary bee *L. (Ctenonomia) NDA-1*, we compared individual excavation performance before and after the bees experienced a social environment. We also examined these artificially constructed social groups for the emergence of division of labour.

METHODS

Study Species

We collected females of the normally solitary *L. (Ctenonomia) NDA-1* in the Cobboboonee State Forest (latitude 141.53 E, longitude 38.19 S, elevation 70 m), Southwestern Victoria, Australia, during December 2006. A previous excavation of *L. (Ctenonomia) NDA-1* revealed that only two of 19 nests contained two females, confirming their status as primarily solitary nesting (McConnell-Garner & Kukuk 1997). We collected bees by netting above nest aggregations and took them immediately to the laboratory, where we placed them individually in petri dishes supplied with a damp tissue and a 1:1 honey:water mixture. The day before the first experiment, we marked two groups of bees with enamel paint on the thorax and both sides of the abdomen. We randomly assigned the bees to two treatments: a 'social' treatment in which bees experienced the presence of a conspecific during a portion of the experiment and a solitary treatment

(hereafter control bees) in which bees did not encounter conspecifics for the duration of the experiment. We stored the bees overnight at ambient temperature (16 °C) before experimental treatment.

Experimental Design

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

Activity level (day 1)

To examine whether differences in the nature of social interactions or task performance may be influenced by differences in an individual's general activity level, we placed all bees individually into a circular plastic tube (inner diameter 0.42 cm, length 20 cm) and quantified the amount of time they were active (Fig. 1). The circular tube was placed in a plastic box with long tubular lights on each side to ensure homogeneous lighting during data collection. The temperature was kept at 27 ± 0.5 °C. Each trial began 2 min after the bee was introduced into the box and lasted for 5 min. During trials we recorded the percentage of time a bee was walking or sitting/grooming and measured the time that individuals took to cover a distance of 10 cm without stopping or turning. This experiment took place between 0930 and 1300 hours.

Tunnel construction by single individuals (days 1–2)

After assessment of activity levels, we introduced all bees individually (between 1400 and 1500 hours) into observation nests (15 cm deep \times 12 cm wide \times 3 mm thick) and allowed them to excavate tunnels. Observation nests were filled with dampened sifted soil collected from the nesting area in the field. Each nest was connected by a clear plastic tube (4 cm) to a clear plastic vial (2 cm diameter, 4 cm high) containing a 1:1 honey:water mixture. We made a small indentation of 1 ± 0.1 cm in the soil to encourage the bees to dig a single vertical gallery. Prior to introduction of the bees, we cooled the nests to 5 °C. After introducing the bees into the nests, we placed the nests in a climate-controlled room at 1500 hours. The climate-controlled room was kept at 31 ± 1 °C with a photoperiod of 15 h of light and 9 h of darkness; darkness started at 1900 hours. We allowed the bees to excavate for 18 h.

At 0900 hours on day 2, we removed the bees from the observation nest and placed them back in their petri dishes. Using a ruler, we measured the length of each bee's tunnel to quantify their digging performance and assign them to treatment groups. We also digitally photographed each nest to quantify more precisely the tunnel depths using ImageJ (see below).

Social interactions (day 2)

We randomly assigned the bees to either the solitary ($N = 30$ bees) or the social treatment group ($N = 35$ pairs). We paired bees assigned to the social treatment using individual digging performance data. To minimize initial interindividual differences in propensity to dig, we matched individuals with similar digging performance (the two bees that dug the most were paired, and so on, until the two bees that dug the least were paired). Prior to placing them together in a nest, we introduced each pair into a circular plastic tube, under the same light and temperature conditions as for activity levels, to quantify their social interactions (see below) (Fig. 1). Observations began 2 min after the bees were introduced and lasted 7 min. We observed three types of interindividual

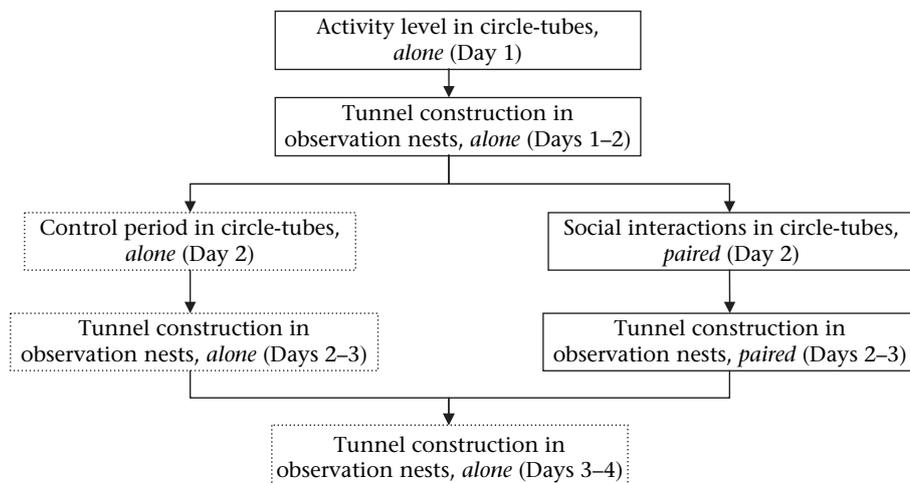


Figure 1. Sequence of experimental design.

encounters in the social assays. In all cases, the interindividual distance of the two bees was less than 2 mm when these encounters were recorded.

(1) Head-to-head encounter (a frontal encounter).

(2) Head-to-back encounter (a forward-moving bee encountered the back of a sitting bee).

(3) Back-to-back encounter (one or both bees backed up in the tube and encountered the other).

Once contact was established, we recorded the outcomes of the interactions, including the following.

(1) U-turn to head to head (following a head-to-back encounter, one bee made a U-turn, resulting in a head-to-head encounter).

(2) Withdraw (one bee made a U-turn and moved away from the other).

(3) Back (one bee backed away after encountering the other).

(4) C-posture (a bee curled her abdomen, giving a C-shaped position with the sting pointed at the other female).

(5) Bite (one bee bit the other).

(6) Lunge (one bee quickly lunged towards the other).

(7) Block (one bee curled the abdomen to prevent the other bee from passing).

(8) Pass (bees passed by each other, requiring the rotation of each bee while moving past the other, venter to venter; we identified the bee initiating the pass, the bee allowing the pass and instances in which both bees mutually passed).

(9) No response (sitting and/or self-grooming).

We introduced control bees individually into the circular tube under the same light and temperature conditions for 9 min, but because they were alone, we could not assess their social behaviour. This experiment took place between 1000 and 1230 hours on day 2.

Tunnel construction by single and paired individuals (days 2–3)

After the circle-tube assays for social interactions, we placed each solitary treatment bee and each pair from the social treatment into different observation nests. We kept the bees in the same pairs for circle-tube assays and observation nests. We performed four sessions of 20 scans each every 6 h over the next 18 h (240 scans in total). We scanned individual nests every 5 min. During each scan we recorded the location and behaviours of each bee, following Jeanson *et al.* (2005). Behaviours consisted of the following.

(1) Excavate (the bee excavated dirt with her mandibles).

(2) Push (the bee pushed loose soil in the tunnel towards the nest entrance or vial).

(3) Tamp (the bee tamped loose dirt into side walls with her abdomen; *Batra 1964*).

(4) Gate-keep (the bee sat in the tube connecting the nest and the vial).

We also recorded all instances of sitting, grooming, walking and feeding from the honey-water solution in the vial. These behaviours either were not associated with specific tasks or were rarely performed (feeding) and thus were not used in

division of labour or task specialization analyses. The tasks of pushing and tamping generally occurred together and so were combined for analyses.

We categorized bee locations as vial, tube, tunnel, bottom (dead end of a gallery). The three focal tasks were spatially segregated within the nest; excavation occurred at the bottom of a gallery, pushing/tamping took place in the tunnel or in the tube and gate-keeping occurred close to the nest entrance. Excavation could be performed by only one bee at a time within a tunnel, but pushing/tamping and gate-keeping could be performed by both bees simultaneously. After 18 h, we digitally photographed the nests to measure the lengths of the tunnels.

Tunnel construction by single individuals (days 3–4)

To determine whether individual response thresholds changed with experience, we removed bees from their paired (social treatment) or solitary (control treatment) nests and transferred them individually into new observation nests at 1500 hours on day 3. After 18 h, we removed bees and measured the total tunnel length of each nest. At the end of the experiments, we killed the bees and preserved them in 95% ethanol.

Data Analysis

Tunnel length

We photographed each nest with a digital camera. From the pictures, we traced the tunnels using the software ImageJ (<http://rsb.info.nih.gov/ij>) to measure their total length precisely. There was a strong correlation between the total length and the area of the tunnels (Pearson correlation test: $r_{86} = 0.92$, $P < 0.001$). Because there was no variation in the width of tunnels (which equalled approximately the width of a bee), we subsequently compared excavation performance across treatments with reference to tunnel length. For the subsequent analysis, we subtracted 0.9 cm from the length measured to take into account the indentation made in the observation nests.

Number of excavation bouts

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

days 3–4 by multiplying her tunnel length by the average digging rate.

Quantification of division of labour

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

Statistical analysis

We compared the daily intensity of division of labour using repeated-measures ANOVA. We used a MANOVA after square root transformation to compare the number of behavioural bouts performed by pairs and control bees on days 2–3. We examined the excavation performance over days between control bees and individuals within pairs by fitting linear mixed-effects models (lme procedure, Pinheiro & Bates 2000) after square root transformation of the number of excavation bouts. We started with a full model including time (days), group (control, HFE, LFE; see below) and their first- and second-term interactions as fixed effects. Observation nests and days were set as random factors. From the full model, the minimal model was obtained by successively deleting nonsignificant terms using the parsimony principle based on the Akaike information criterion (Pinheiro & Bates 2000).

We compared the number of behavioural bouts performed individually on days 2–3 using t tests after square root transformation. We used Pearson correlation tests to test the relationship between tunnel length across successive days, the relationship between the probability of passing and the intensity of division of labour, and the relationship between activity level and excavation performance. Statistical tests were two tailed and performed with SPSS (version 11.0, SPSS, Inc., Chicago, Illinois, U.S.A.), except linear mixed models, which were done using the statistical package R (R Development Core Team 2008).

RESULTS

Task Performance in Social versus Solitary Contexts

We compared performance rates of solitary and paired bees for the three tasks, excavation, pushing/tamping and

gate-keeping, on days 2–3. Bees in the social treatments collectively performed more behavioural bouts than control bees (Fig. 2). Pairs performed approximately twice the number of excavations (MANOVA: $F_{1,62} = 15.89$, $P < 0.001$) and push/tamp bouts (MANOVA: $F_{1,62} = 28.50$, $P < 0.001$) as control bees. Pairs also displayed about seven times more gate-keeping than control bees (MANOVA: $F_{1,62} = 106.44$, $P < 0.001$). The total per capita task performance was about 1.6 times higher for pairs than for control bees (t test: $t_{96} = 4.62$, $P < 0.001$) and paired bees performed individually about 3.5 times more gate-keeping than control bees (t test: $t_{96} = 4.96$, $P < 0.001$).

Differentiation within Pairs for Excavation Behaviour

Within each pair, we identified a higher frequency excavator (hereafter HFE) as the bee performing more excavation bouts than her nestmate (hereafter LFE, lower frequency excavator). We discarded one pair from this analysis because neither bee dug. In two pairs, because both bees displayed the same number of excavation bouts, we randomly assigned one bee as the HFE. Note that the first bee to be introduced into the nest was not more likely to become the HFE (chi-square test: $\chi_1 = 0.13$, $P = 0.72$).

We used linear mixed models to examine the variations in excavation performance between HFE, LFE and control bees over time (Table 1). There was no difference in the number of excavation bouts performed by control, HFE and LFE bees on days 1–2 (Fig. 3). There was marginally significant variation in the excavation rates of control bees across the 3 days of the experiment. When bees were paired together on days 2–3, their behaviour diverged, such that one bee (HFE) excavated more than the other, and LFE bees maintained an excavation performance similar to that on days 1–2. We separated the pairs on day 3 and reassessed individual excavation behaviour in isolation. When placed alone in observation nests on days 3–4, LFE bees dug more than they had while alone on days 1–2 or in pairs on days 2–3. In contrast, HFE

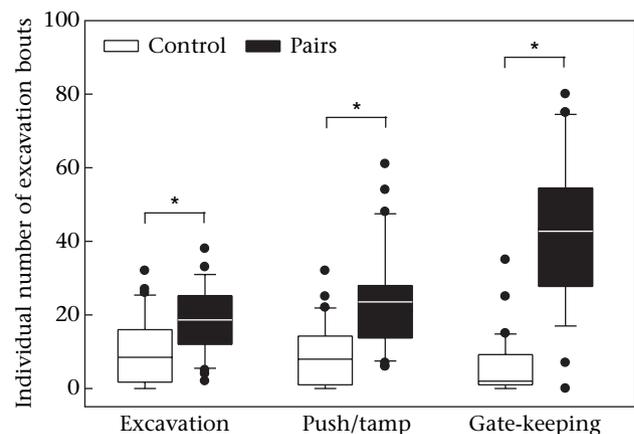


Figure 2. Box plots of the total numbers of excavation, push/tamp and gate-keeping bouts performed by control bees and pairs on days 2–3. *Indicates statistical significance. Boxes show median value, 25th and 75th percentiles and outliers.

Table 1. Results of the linear mixed model fitted by maximum likelihood for the square root of the number of excavation bouts performed individually

	Estimate	SE	df	<i>t</i> value	<i>P</i>
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

Parameter estimates (i.e. fixed-effects estimates), approximate standard errors, degrees of freedom, *t* estimates (ratio between the estimates and the standard errors) and *P* values are given. Control represents bees placed individually into observation nests. HFE (higher frequency excavator) and LFE (lower frequency excavator) represent bees within pairs; each bee within a pair was designated as HFE or LFE based on the number of observed times she excavated while in the pair. Nest refers to observation nest. Formulae follow the convention of the R language. Random effects formula: ~ days|nests; standard deviation: intercept = 1.37, days = 0.54, residual = 1.35. Fixed effects formula: $\sqrt{(\text{individual excavation bouts})} \sim (\text{group} \times \text{days}) + (\text{group} \times \text{days}^2)$.

bees excavated more on days 2–3 and on days 3–4 than on days 1–2 but their performance did not differ between days 2–3 and days 3–4. The tunnels dug by HFE and LFE on days 3–4 were, respectively, 1.65 and 2.5 times longer than on days 1–2.

Task Specialization and Level of Division of Labour in Pairs

Bees designated as HFE and LFE for excavation behaviour showed differential performance of other tasks while together (*G* test: $G_2 = 10.56$, $P < 0.01$). There were no differences between HFE and control (*t* test: $t_{62} = 1.9$, $P = 0.06$), LFE and control (*t* test: $t_{62} = 0.06$, $P = 0.54$) or LFE and HFE in their pushing/tamping behaviour (paired

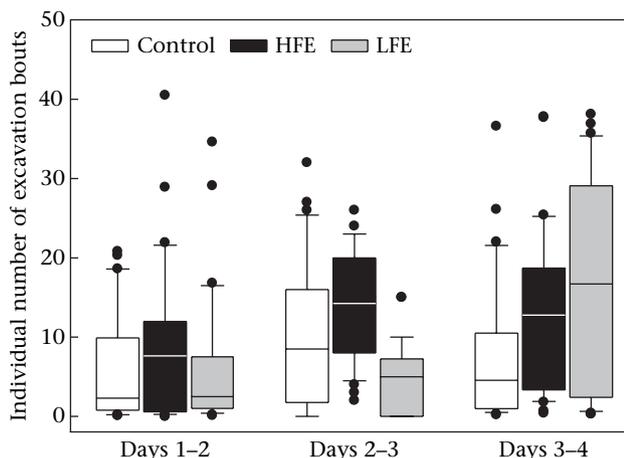


Figure 3. Box plots of excavation bouts performed daily by control, HFE (higher frequency excavator, i.e. bee performing more excavation bouts than her nestmate within pairs) and LFE (lower frequency excavator) bees. Boxes show median value, 25th and 75th percentiles and outliers.

t test: $t_{33} = 1.22$, $P = 0.23$; for all tests, corrected $\alpha = 0.017$) (Fig. 4). Both LFE (*t* test: $t_{62} = 6.33$, $P < 0.001$) and HFE (*t* test: $t_{62} = 3.12$, $P = 0.003$) displayed significantly more gate-keeping bouts than did control bees. The LFE bees also displayed significantly more gate-keeping bouts than HFE bees (paired *t* test: $t_{33} = 3.03$, $P = 0.005$; for all tests, corrected $\alpha = 0.017$).

For each pair, we computed the DOL metric for each session of 20 scans during the 18 h period on days 2–3. One pair did not dig during days 2–3 and was excluded from the analysis. For the three focal tasks, the total performance of each pair equalled at least 36 bouts (median 84, first quartile 67, third quartile 101) and each bee within a pair performed at least 11 bouts (median 54, first quartile 31, third quartile 54). Division of labour did not change over time (repeated-measures ANOVA: $F_{3,93} = 0.27$, $P = 0.85$); therefore we pooled the data for analysis. Across the four sessions of 20 scans (80 scans per pair), the average $\text{DOL}_{\text{tasks} \rightarrow \text{indiv}}$ was 0.26 ± 0.04 ($N = 34$; median 0.13, first quartile 0.09, third quartile 0.45). There was no correlation between tunnel length and $\text{DOL}_{\text{tasks} \rightarrow \text{indiv}}$ (Pearson correlation test: $r_{34} = -0.28$, $P = 0.10$).

Activity Levels

Control and paired bees did not differ in the amount of time they spent walking when they were alone in the circle tubes (control: $\bar{X} \pm \text{SEM} = 0.37 \pm 0.06$; paired bees: 0.34 ± 0.03 ; *t* test: $t_{98} = -0.47$, $P = 0.64$). For paired bees, there was no correlation between individual activity level (time spent walking) and the length of their tunnel dug alone on days 1–2 or on days 3–4 (Pearson correlation: days 1–2: $r_{70} = 0.09$, $P = 0.44$; days 3–4: $r_{70} = -0.18$, $P = 0.14$). Likewise, there was no correlation between activity level and tunnel length on any of the 3 days for control bees (Pearson correlation days 1–2: $r_{30} = 0.07$, $P = 0.71$; days 2–3: $r_{30} = 0.33$, $P = 0.07$; days 3–4:

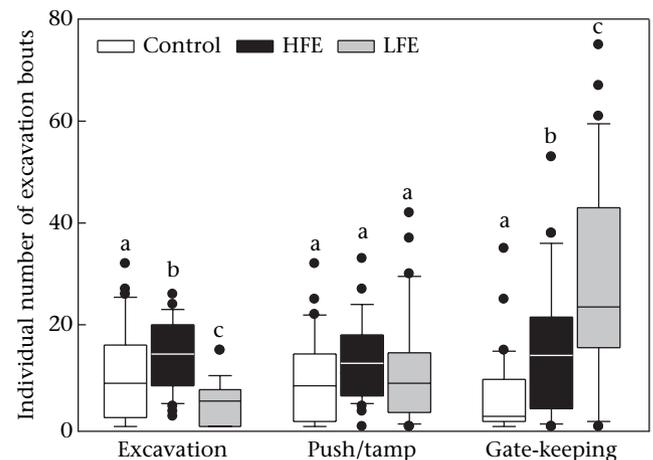


Figure 4. Box plots of the number of behavioural bouts performed by control, HFE (higher frequency excavator) and LFE (lower frequency excavator) bees on days 2–3. For each behaviour, different letters indicate significant statistical differences. Boxes show median value, 25th and 75th percentiles and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

$r_{30} = 0.16$, $P = 0.39$). Activity level did not predict which bees would subsequently be categorized as HFE or LFE (HFE: $\bar{X} \pm \text{SEM} = 0.32 \pm 0.05$; LFE: 0.39 ± 0.05 ; t test: $t_{66} = -1.18$, $P = 0.24$).

Social Interactions

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

DISCUSSION

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

In this study, bees were paired so as to minimize the initial interindividual differences in their propensity to dig. However, once in pairs, the bees altered their behaviours. One bee specialized in excavation and performed about two times more excavation bouts than when she was previously alone. In contrast, her nestmate decreased her excavation performance while increasing her gate-keeping. These data support the assertion that division of labour can spontaneously emerge from social dynamics, even at the origins of sociality. The differentiation between bees that were similar in behaviour before they were placed in social groups indicates that the observed task specialization is indeed an effect of social dynamics and not simply a reflection of individual variation within the group (Fewell & Page 1999).

Similar patterns of task specialization have been found in associations of normally solitary foundresses of the harvester ant *Pogonomyrmex californicus* (Helms Cahan & Fewell 2004; Jeanson & Fewell 2008).

Self-reinforcement and Task Differentiation

Under the hypothesis of self-reinforcement, the more an individual performs a task, the lower its associated threshold becomes (Theraulaz et al. 1998). The excavation specialist (HFE) was thus expected to display subsequently a higher propensity to dig after taking over the task of excavation in pair groups. In our experiments, the HFE indeed performed more excavation on days 3–4 than on days 1–2, after they dug intensively on days 2–3. However, LFE performed the task even more frequently after experiencing the social context. Indeed, the excavation performance of LFE was similar on days 1–2 and days 2–3 but dramatically increased (by a factor of 2.5) on days 3–4 and reached about 85% of the performance achieved by the HFE specialists on days 2–3. Conversely, bees kept alone varied little in their excavation performance across days. Although self-reinforcement might account for the increased performance of excavating specialists, it is unlikely to explain the enhanced level of digging among nonspecialist bees. The increased excavation behaviour of both specialists and nonspecialists compared with their excavation behaviour prior to being paired suggests that the earlier social context experienced by bees induced a delayed modulation of response threshold for excavation that cannot be explained by self-reinforcement alone or any other existing models of task performance and/or division of labour. In addition, division of labour did not increase during the first 18 h of nest construction, contrary to the hypothesis that self-reinforcement amplifies behavioural differentiation over time.

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

Social Modulation of Excavation Behaviour

Although our data do not support self-reinforcement as the primary mechanism driving task differentiation, they clearly show that excavation is socially modulated; both

bees showed a reduction in their individual response thresholds for excavation after they experienced the presence of a nestmate. A different pattern has been documented in associations of normally social individuals. For instance, the ant *Camponotus japonicus* digs significantly larger amounts of sand in associations than when the same ants work separately but does not alter individual digging performance before and after being paired (Chen 1937a, b). Similarly, in social halictine bees such as the communal *L. (Chilalictus) hemichalceum* (Jeanson et al. 2005) and the eusocial *L. zephyrum* (Michener et al. 1971), individuals that experience repeated contact show enhanced digging activity over bees that are housed without social contact. An increase in the propensity to express a behaviour when others express it is known as social facilitation (Clayton 1978; Webster & Fiorito 2001). Social facilitation could be attributed to a reduction in the individual response thresholds associated with a specific task depending on the perception of conspecifics already engaged in the same activity. A comparative study between populations with different social structures should aid in our understanding of how social context-induced modifications in individual response thresholds may have paralleled social transitions in halictine bees. In particular, we predict that communal bees kept alone should have higher individual response thresholds for excavation than solitary bees because repeated social contact seems to be mutually stimulating within social populations.

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

investigate whether modifications of individual response thresholds correlate with variations in amine titres depending on social context.

Aggression and Task Allocation

Aggression and dominance interactions can contribute to the establishment of reproductive division of labour in both invertebrates (e.g. ants, Monnin & Peeters 1999; wasps, Sledge et al. 2001) and vertebrates (e.g. spotted hyenas, Creel et al. 1997). Aggressive interactions among eusocial insect workers can also regulate allocation of non-reproductive tasks. In the wasp *Polybia occidentalis*, biting interactions among workers modulate the probability of foraging (O'Donnell 2003, 2006). In the present study, the initiator or recipient of aggressive acts (e.g. C-posture) was not more likely to specialize in excavation or guarding within pairs, and division of labour apparently did not emerge through social contention. However, this does not mean that social interactions did not contribute to task specialization. Indeed, Jeanson et al. (2005) found that the low probability of passing in *L. NDA-1* generated a spatial segregation of solitary bees within the nest, which consequently reduced the turnover among tasks and indirectly reinforced task asymmetry with pairs (Jeanson et al. 2005).

Guarding in Solitary and Social Contexts

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

Although there was no possibility of predators or other intruders in our experiment, gate-keeping is behaviourally similar to guarding in a natural context. In many halictine species, the nest consists of a burrow that enters the soil, with lateral cells that are provisioned with pollen and nectar (Michener 1964). The entrance of the nest is generally constricted and fits the head or abdomen size of a bee. Bees guard the nest by blocking the nest entrance with their head or by presenting their abdomen (Batra 1964; Knerer 1969). In the halictine bee *Agapostemon virescens*, guarding is responsible for the accrued resistance to attack by kleptoparasites in communal nests in comparison to

solitary nesting (Abrams & Eickwort 1981). Abrams & Eickwort (1981) proposed that social nesting permits bees to (1) guard nests at all times; (2) conserve digging effort per nestmate; (3) dig deeper burrows, reaching soil levels with optimal water content. During the incipient stages of sociality, associations of initially solitary individuals may therefore have benefited from improved nest defence through increased guarding and improved ability to reach soils with optimal water content. These benefits may have promoted transitions to communal sociality in halictine bees.

Acknowledgments

We thank the National Science Foundation for Integrative Organismal Systems Collaborative Research Grant 0446415 supporting this research. We thank P. Michelena and C. Andalo for useful discussions. S.M.B. also thanks Carleton University for partial support through start-up funds.

References

- Abrams, J. & Eickwort, G. C. 1981. Nest switching and guarding by the communal sweat bee *Agapostemon virescens* (Hymenoptera, Halictidae). *Insectes Sociaux*, **28**, 105–116.
- Barron, A. B. & Robinson, G. E. 2005. Selective modulation of task performance by octopamine in honey bee (*Apis mellifera*) division of labour. *Journal of Comparative Physiology A—Neuroethology Sensory Neural and Behavioral Physiology*, **191**, 659–668.
- Barron, A. B., Schulz, D. J. & Robinson, G. E. 2002. Octopamine modulates responsiveness to foraging-related stimuli in honey bees (*Apis mellifera*). *Journal of Comparative Physiology A—Neuroethology Sensory Neural and Behavioral Physiology*, **188**, 603–610.
- Batra, S. W. T. 1964. Behavior of the social bee, *Lasioglossum zephyrum*, within the nest (Hymenoptera, Halictidae). *Insectes Sociaux*, **2**, 159–186.
- Bednarz, J. C. 1988. Cooperative hunting in Harris' hawks. *Science*, **239**, 1525–1527.
- Bennett, N. C. & Faulkes, C. G. 2000. *African Mole-Rats: Ecology and Eusociality*. Cambridge, U.K.: Cambridge University Press.
- Beshers, S. N. & Fewell, J. H. 2001. Models of division of labor in social insects. *Annual Review of Entomology*, **46**, 413–440.
- Bonabeau, E., Theraulaz, G. & Deneubourg, J.-L. 1996. Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proceedings of the Royal Society of London, Series B*, **263**, 1565–1569.
- Bonabeau, E., Theraulaz, G. & Deneubourg, J. L. 1998. Fixed response thresholds and the regulation of division of labor in insect societies. *Bulletin of Mathematical Biology*, **60**, 753–807.
- Breed, M. D., Siverman, J. M. & Bell, W. J. 1978. Agonistic behavior, social interactions, and behavioral specialization in a primitively eusocial bee. *Insectes Sociaux*, **25**, 351–364.
- Camazine, S., Deneubourg, J. L., Franks, N. R., Sneyd, J., Theraulaz, G. & Bonabeau, E. 2001. *Self-Organization in Biological Systems*. Princeton, New Jersey: Princeton University Press.
- Chen, S. C. 1937a. Social modification of the activity of ants in nest-building. *Physiological Zoology*, **10**, 420–436.
- Chen, S. C. 1937b. The leaders and followers among the ants in nest-building. *Physiological Zoology*, **10**, 437–455.
- Clayton, D. A. 1978. Socially facilitated behavior. *Quarterly Review of Biology*, **53**, 373–392.
- Creel, S., Creel, N. M., Mills, M. G. L. & Monfort, S. L. 1997. Rank and reproduction in cooperatively breeding African wild dogs: behavioral and endocrine correlates. *Behavioral Ecology*, **8**, 298–306.
- Crespi, B. J. 1992. Eusociality in Australian gall thrips. *Nature*, **359**, 724–726.
- Cuvillier-Hot, V. & Lenoir, A. 2006. Biogenic amine levels, reproduction and social dominance in the queenless ant *Streblognathus peetersi*. *Naturwissenschaften*, **93**, 149–153.
- Deneubourg, J.-L., Goss, S., Pasteels, J. M., Fresneau, D. & Lachaud, J.-P. 1987. Self-organization mechanisms in ant societies (2): learning in foraging and division of labor. In: *From Individual to Collective Behavior in Social Insects* (Ed. by J. M. Pasteels & J. L. Deneubourg), Basel: Birkhäuser-Verlag.
- Duffy, J. E., Morrison, C. L. & Macdonald, K. S. 2002. Colony defense and behavioral differentiation in the eusocial shrimp *Synalpheus regalis*. *Behavioral Ecology and Sociobiology*, **51**, 488–495.
- Dunn, T. & Richards, M. H. 2003. When to be social: interactions among environmental constraints, incentives, guarding, and relatedness in a facultatively social carpenter bee. *Behavioral Ecology*, **14**, 417–424.
- Fewell, J. H. & Page, R. E. 1999. The emergence of division of labour in forced associations of normally solitary ant queens. *Evolutionary Ecology Research*, **1**, 537–548.
- Fewell, J. H. & Page, R. E. 2000. Colony-level selection effects on individual and colony foraging task performance in honeybees, *Apis mellifera* L. *Behavioral Ecology and Sociobiology*, **48**, 173–181.
- Gazda, S. K., Connor, R. C., Edgar, R. K. & Cox, F. 2005. A division of labour with role specialization in group-hunting bottlenose dolphins (*Tursiops truncatus*) off Cedar Key, Florida. *Proceedings: Biological Sciences*, **272**, 135–140.
- Gerber, C., Badertscher, S. & Leuthold, R. H. 1988. Polyethism in *Macrotermes bellicosus* (Isoptera). *Insectes Sociaux*, **35**, 226–240.
- Gorelick, R., Bertram, S. M., Killeen, P. R. & Fewell, J. H. 2004. Normalized mutual entropy in biology: quantifying division of labor. *American Naturalist*, **164**, 678–682.
- Grasmuck, V. & Desor, D. 2002. Behavioural differentiation of rats confronted to a complex diving-for-food situation. *Behavioural Processes*, **58**, 67–77.
- Helms Cahan, S. & Fewell, J. H. 2004. Division of labor and the evolution of task sharing in queen associations of the harvester ant *Pogonomyrmex californicus*. *Behavioral Ecology and Sociobiology*, **56**, 9–17.
- Hogendoorn, K. & Schwarz, M. P. 1998. Guarding specialisation in pre-reproductive colonies of the allodapine bee *Exoneura bicolor*. *Ethology Ecology & Evolution*, **10**, 67–77.
- Hogendoorn, K. & Velthuis, H. H. W. 1999. Task allocation and reproductive skew in social mass provisioning carpenter bees in relation to age and size. *Insectes Sociaux*, **46**, 198–207.
- Hölldobler, B. & Wilson, E. O. 1990. *The Ants*. Cambridge, Massachusetts: Belknap Press of Harvard University Press.
- Huber, R., Panksepp, J. B., Yue, Z., Delago, A. & Moore, P. 2001. Dynamic interactions of behavior and amine neurochemistry in acquisition and maintenance of social rank in crayfish. *Brain Behavior and Evolution*, **57**, 271–282.
- Jeanson, R. & Fewell, J. H. 2008. Influence of the social context on division of labor in ant foundress associations. *Behavioral Ecology*, **19**, 567–574.
- Jeanson, R., Kukuk, P. F. & Fewell, J. H. 2005. Emergence of division of labour in halictine bees: contributions of social interactions and behavioural variance. *Animal Behaviour*, **70**, 1183–1193.
- Jones, J. C., Myerscough, M. R., Graham, S. & Oldroyd, B. P. 2004. Honey bee nest thermoregulation: diversity promotes stability. *Science*, **305**, 402–404.

- Knerer, G. 1969. Synergistic evolution of halictine nest architecture and social behavior. *Canadian Journal of Zoology*, **47**, 925–930.
- McConnell-Garner, J. & Kukuk, P. F. 1997. Behavioral interactions of two solitary, halictine bees with comparisons among solitary, communal and eusocial species. *Ethology*, **103**, 19–32.
- Michener, C. D. 1964. Evolution of the nests of bees. *American Zoologist*, **4**, 227–239.
- Michener, C. D., Brothers, D. J. & Kamm, D. R. 1971. Interactions in colonies of primitively eusocial bees: artificial colonies of *Lasiosglossum zephyrum*. *Proceedings of the National Academy of Sciences*, **68**, 1241–1245.
- Monnin, T. & Peeters, C. 1999. Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. *Behavioral Ecology*, **10**, 323–332.
- O'Donnell, S. 2003. The development of biting interactions and task performance in a tropical eusocial wasp. *Behaviour*, **140**, 255–267.
- O'Donnell, S. 2006. Polybia wasp biting interactions recruit foragers following experimental worker removals. *Animal Behaviour*, **71**, 709–715.
- O'Donnell, S. & Foster, R. L. 2001. Thresholds of response in nest thermoregulation by worker bumble bees, *Bombus bifarius nearcticus* (Hymenoptera: Apidae). *Ethology*, **107**, 387–399.
- Oster, G. F. & Wilson, E. O. 1978. *Caste and Ecology in the Social Insects*. Princeton, New Jersey: Princeton University Press.
- Patterson, C. J., Sutfin, E. L. & Fulcher, M. 2004. Division of labor among lesbian and heterosexual parenting couples: correlates of specialized versus shared patterns. *Journal of Adult Development*, **11**, 179–189.
- Pflüger, H. J. & Stevenson, P. A. 2005. Evolutionary aspects of octopaminergic systems with emphasis on arthropods. *Arthropod Structure & Development*, **34**, 379–396.
- Pinheiro, J. C. & Bates, D. M. 2000. *Mixed-Effects Models in S and S-PLUS*. New York: Springer-Verlag.
- Plowright, R. C. & Plowright, C. M. S. 1988. Elitism in social insects: a positive feed-back model. In: *Interindividual Behavioral Variability in Social Insects* (Ed. by R. L. Jeanne), pp. 419–431. Boulder, Colorado: Westview Press.
- Punzo, F. & Punzo, T. 2001. Monoamines in the brain of tarantulas (*Aphonopelma hentzi*) (Araneae, Tetranychidae): differences associated with male agonistic interactions. *Journal of Arachnology*, **29**, 388–395.
- Ravary, F., Lecoutey, E., Kaminski, G., Chaline, N. & Jaisson, P. 2007. Individual experience alone can generate lasting division of labor in ants. *Current Biology*, **17**, 1308–1312.
- R Development Core Team. 2008. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Robinson, G. E. & Page, R. E., Jr. 1988. Genetic determination of guarding and undertaking in honey-bee colonies. *Nature*, **333**, 356–358.
- Robinson, G. E. & Page, R. E., Jr. 1989. Genetic basis for division of labor in an insect society. In: *The Genetics of Social Evolution* (Ed. by M. D. Breed & R. E. Page Jr), pp. 61–80. Boulder, Colorado: Westview Press.
- Sakagami, S. F. & Maeta, Y. 1987. Sociality, induced and/or natural, in the basically solitary small carpenter bees (*Ceratina*). In: *Animal Societies: Theories and Facts* (Ed. by Y. Itô, J. L. Brown & L. Kikkawa), pp. 1–16. Tokyo: Japan Scientific Societies Press.
- Scheiner, R., Pluckhahn, S., Oney, B., Blenau, W. & Erber, J. 2002. Behavioural pharmacology of octopamine, tyramine and dopamine in honey bees. *Behavioural Brain Research*, **136**, 545–553.
- Sherman, P. W., Jarvis, J. U. M. & Alexander, R. D. 1991. *The Biology of the Naked Mole-Rat*. Princeton, New Jersey: Princeton University Press.
- Sledge, M. F., Boscaro, F. & Turillazzi, S. 2001. Cuticular hydrocarbons and reproductive status in the social wasp *Polistes dominulus*. *Behavioral Ecology and Sociobiology*, **49**, 401–409.
- Stander, P. E. 1992. Cooperative hunting in lions: the role of the individual. *Behavioral Ecology and Sociobiology*, **29**, 445–454.
- Stevenson, P. A., Dyakonova, V., Rillich, J. & Schildberger, K. 2005. Octopamine and experience-dependent modulation of aggression in crickets. *Journal of Neuroscience*, **25**, 1431–1441.
- Stuart, R. J. & Page, R. E. 1991. Genetic component to division of labor among workers of a Leptoracine ant. *Naturwissenschaften*, **78**, 375–377.
- Theraulaz, G., Bonabeau, E. & Deneubourg, J. L. 1995. Self-organization of hierarchies in animal societies—the case of the primitively eusocial wasp *Polistes dominulus* Christ. *Journal of Theoretical Biology*, **174**, 313–323.
- Theraulaz, G., Bonabeau, E. & Deneubourg, J.-L. 1998. Response threshold reinforcement and division of labour in insect societies. *Proceedings of the Royal Society of London, Series B*, **265**, 327–332.
- Underwood, D. L. A. & Shapiro, A. M. 1999. Evidence for division of labor in the social caterpillar *Eucheira socialis* (Lepidoptera: Pieridae). *Behavioral Ecology and Sociobiology*, **46**, 228–236.
- Webster, S. & Fiorito, G. 2001. Socially guided behaviour in non-insect invertebrates. *Animal Cognition*, **4**, 69–79.
- Weidenmüller, A. 2004. The control of nest climate in bumblebee (*Bombus terrestris*) colonies: interindividual variability and self reinforcement in fanning response. *Behavioral Ecology*, **15**, 120–128.
- Wilson, E. O. 1975. *Sociobiology: the New Synthesis*. Cambridge, Massachusetts: Harvard University Press.