

Does substrate coarseness matter for foraging ants? An experiment with *Lasius niger* (Hymenoptera; Formicidae)

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Abstract

We investigated whether workers of the ant species *Lasius niger* are able to sense and discriminate the coarseness of the substrate on which they walk. First, we studied the way in which substrate coarseness affects the ants' locomotory behaviour. Second, we investigated the spontaneous preference of ants for substrates of different coarseness. And third, we tested with a differential conditioning procedure the ants' capacity to learn to associate a given coarseness with a food reward. The locomotory behaviour of ants differed according to substrate coarseness: ants moved significantly faster and had more sinuous trajectories on a fine than on a coarse substrate. No spontaneous preference for a substrate of a given coarseness was observed and, even after 20 successive conditioning trials, there was little evidence of the effect of experience on substrate coarseness discrimination. Overall however, ants trained on fine sand made significantly more correct choice than those trained on coarse sand. We discuss these results and argue that in *L. niger* substrate coarseness may be more important at the collective level, by interacting with the chemical properties of the pheromone trail used in mass recruitment to food source, than at the individual level.

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1. Introduction

Touch is a sense of paramount importance for insects that are blind or live in darkness, e.g. in natural or built cavities or in underground galleries and chambers. In the absence of vision, insects can inspect food or other objects through their tactile sense and can acquire information about their position, shape, size and texture (Kevan, 1987). Tactile information in insects can be acquired without direct body contact through the stimulation of the tactile hairs located on the cercae (Dangles et al., 2006) or the antennae (Okada and Toh, 2000; Scheiner et al., 2005; Cowan et al., 2006). Touch is also a sense used by insects whose vision is not impaired and it can supplement the information acquired by the other senses to discriminate between objects. For example, workers of the honeybee *Apis mellifera* are able to discriminate between objects of

different surface textures (Kevan and Lane, 1985; Kevan, 1987; Erber et al., 1997, 1998; Scheiner et al., 1999, 2005) or of different shapes (Erber et al., 1997; Scheiner et al., 2005) by scanning them with their antennae.

Touch can also provide precious information on the characteristics of the substrate on which the insects are moving. For example, the use of tactile cues to discriminate between leaf textures in insects ovipositing on the vegetation is well documented (Foster et al., 1997; Foster and Howard, 1998; Kanno and Harris, 2000; Rojas et al., 2003). However, this use of the tactile sense is much less documented in the literature than that involved in the recognition and discrimination of objects. Yet, this information could be important at the moment an insect chooses its habitat or when it orients to specific places in the environment. Duelli and Duelli-Klein (1976) report, for instance, a field experiment on a South American ant species in which workers coming back from a food source had to walk sequentially on different substrates in order to reach their nest. When nestbound ants were captured and

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passively displaced at another point further along a sandy road, they began to make search loops after walking the distance they should have walked on the road before encountering another substrate had they not been displaced by the experimenters. The fact that ants do use the mechanical properties of the substrate to orient in their environment has indeed been recently shown in the species *Cataglyphis fortis* by Seidl and Wehner (2006): when *C. fortis* workers nearing their nest encountered a substrate whose coarseness differed from that they had been used to in previous training sessions they tried to avoid it.

In this paper, we evaluated the importance of substrate coarseness on foraging workers of the black garden ant *Lasius niger*. We chose this species because of its extended geographical range and the diversity of environments in which it can be found. *L. niger* is a monomorph species (size of the workers: 3–4 mm) which mainly forages collectively along pheromone trails (Beckers et al., 1992), but whose workers can also forage individually when they hunt for small prey. *L. niger* can be found from Western Europe to Mongolia and it is particularly abundant in arable land, as well as in cities, parks and gardens (Seifert, 1992). In these environments foraging workers are likely to encounter different substrates (soil, rocks, concrete, grass, leaves and bark) characterized by different degree of coarseness. Along with chemical and visual cues (Carthy, 1951; Aron et al., 1993) substrate coarseness could thus be a cue that workers could use to orient in the environment. We proceeded in three stages to evaluate the importance of substrate coarseness on foraging ants. First, we evaluated the impact of substrate coarseness on the behaviour of the ants through the characterization of their movement pathway. By increasing the coarseness of the substrate we increase the degree of heterogeneity of the environment at the scale of the ants. In that case, we expect that they should decrease their locomotory rate and move along a more meandering path. An increase in the fractal dimension of the paths, i.e. their tortuosity, with the degree of heterogeneity of the environment has indeed been noted in several species of insects, including ants (Wiens et al., 1995). Second, we evaluated the ecological significance of substrate coarseness for naive foraging ants by testing their spontaneous preference in binary choices with substrates of different coarseness. If substrate coarseness has an ecological significance for *L. niger* workers in their natural environment, we expect that they will express a spontaneous preference for a particular substrate. And third, we tested whether substrate coarseness can acquire a significance for foraging ants by testing whether they are able to learn to associate a given substrate coarseness with the presence of a food reward. If ants can acquire a selective advantage in their natural environment by discriminating between substrate coarseness, we expect that they will have evolved this capability through natural selection.

2. Materials and methods

2.1. Ant collection and rearing conditions

Five colonies of *L. niger* were collected on the campus of the University Paul Sabatier (Toulouse, France) during the summer of 2005. They were reared in the laboratory under controlled temperature ($25 \pm 2^\circ\text{C}$) and photoperiod (12:12 L:D) conditions. The five colonies were used as a stock to constitute five standardized experimental groups containing about 500 workers that were housed in a plastic box of 100 mm diameter (nest-box) connected to another circular plastic box that was used as a foraging area. The walls of the boxes were coated with Fluon[®] to prevent ants from escaping. The number of workers in each experimental group was kept constant throughout the experiments by taking ants from the stock colonies to compensate for loss and death. Ants were fed three times a week with a mixed diet of vitamin-enriched food (Bhaktar and Whitcomb, 1970) and maggots.

2.2. Substrate used

Calcareous sand was collected in a quarry near Toulouse and sieved in the laboratory with sieves of different mesh size. We checked the size range of the sand particles obtained with each sieve with a particle size analyser (Scirocco, Mastersizer, Malvern Instruments[®]). Four sands of different sizes were obtained: 400–560, 710–850, 1000–1120 and 1250–1400 μm .

2.3. Experiments

Ants were starved for 4 days before each experiment.

2.3.1. Experiment 1: locomotory behaviour

In the first experiment, we tested whether the locomotory behaviour of the ants is affected by the coarseness of the substrate on which they walk. Freely foraging workers moving either on fine (400–560 μm) or on coarse (1250–1400 μm) sand were tracked and their trajectories were analysed.

2.3.1.1. Experimental procedure. Each replicate of the experiment consisted in two familiarization trials with the experimental set-up, followed by one test trial.

At the beginning of a replicate an ant chosen randomly in the foraging area of one of the five experimental groups was marked with a colour spot on the thorax. A toothpick was then presented to the marked ant in the foraging arena. As soon as it had climbed on it, the ant was passively transported to a circular test arena (a plastic box of 20 cm diameter) whose walls were coated with Fluon[®] and which was lighted from above by four white neon lights. The arena that was used in the two familiarization trials was empty, while that used in the test trial had its floor covered by a 5 mm layer of either fine or coarse sand. The sand was not fixed to the underlying substrate and it was levelled to

obtain a regular surface. The sand particle analyser shows that the sand grains were more oval than spherical. Therefore, one can assume that the sand layer was stable when an ant walked on it. To homogenize the light and to mask any visual cues that could influence the ant's trajectory, the arenas were surrounded by a white cardboard cylinder. A camera (SONY® DCR-VX200E) placed above the centre of the arena allowed to film the ant during the experiments.

During the two familiarization trials, three droplets of 5 µl of 1 M sucrose solution were used to stimulate the exploratory behaviour of the ant in the subsequent test trial. They were located in each of the 120° sectors of the test arena, at a distance of 5 cm from its centre. The familiarization trial began when the toothpick with the ant on it was inserted vertically in a tiny hole pierced in the centre of the test arena. As soon as the ant had climbed down on the arena floor, the toothpick was removed. Once the ant had discovered a food source, the toothpick was presented to her and we waited until it climbed on it. It was then transported back on the toothpick to the foraging area of its experimental group. After the ant had performed trophallaxis with its nestmates a second familiarization trial was performed. The arena floor was cleaned with 95° alcohol between the two familiarization trials.

The same procedure as for the familiarization trials was applied in the test trial to transport the ant from the foraging area to the test arena. During the test trial however, no food sources were placed in the arena. The ant's exploratory trajectory was filmed during 120 s.

After the completion of the test the ant was placed apart from its experimental group to prevent being reused in another experiment. Twenty-five ants, five each experimental group, were tested for each sand coarseness. The sand of the test arena was changed for every 10 ants. All experiments were run at 25 ± 2 °C.

2.3.1.2. Trajectory acquisition and analysis. We used an automatic tracking software (Ethovision® version 3.0, Noldus Information Technology, <http://www.noldus.com>) to record the paths of the ants at a rate of 5 points per second. For the purpose of the trajectory analysis, the arena was divided into two zones: a central zone (diameter: 18 cm) and a peripheral zone (width: 2 cm). To avoid edge effects, only the parts of the trajectories performed in the central zone were analysed.

The following variables were computed for each trajectory (see Challet et al., 2005):

- The linear speed, i.e. the total distance travelled divided by the total time moving, in cm/s.
- The mean free path, i.e. the mean straight distance walked by the ant, expressed in cm, before a significant directional change (arbitrarily fixed at 5°) occurs. To eliminate the artefacts due to the automatic digitizing process, free paths of length less than 10 mm were not considered.

- The coefficient of directionality: this coefficient characterizes the distribution of directional changes. It gives an estimation of the ant's forward tendency. A value close to +1 indicates that the ant has a high tendency to move forward, whereas a value close to 0 indicates that its trajectory is very sinuous.

2.3.1.3. Statistical analysis. To analyse the effect of the sand coarseness on locomotory behaviour, we performed a multivariate analysis of variance (MANOVA) with linear speed, mean free path and coefficient of anisotropy as dependent variables and sand coarseness (two modalities: 400–560 or 1250–1400 µm) and experimental groups (five) as independent variables.

2.3.2. Experiment 2: spontaneous preference

In this experiment, we tested whether ants express a significant spontaneous preference for a specific substrate coarseness when they are given the choice between two foraging substrates of different coarseness.

2.3.2.1. Experimental procedure. Ants were tested in a PVC rectangular channel (length × width × height: 140 × 25 × 20 mm) whose walls were coated with Fluon®. The vision of *L. niger* workers is known to be reduced in red light (Depickère et al., 2004). Therefore, in order to minimize the use of visual cues in the discrimination of substrate coarseness, the set-up was placed in a black cardboard box and it was lighted from above by a red black chamber bulb (Philips, B22PF712B, 15 W, 5 lx, emission spectrum 600–1000 nm). A hole in the upper part of the box allowed to film the ants during the experiments. Each replicate consists of two familiarization trials with the experimental set-up followed by one test trial.

In the two familiarization trials, the channel was empty and its bottom thus consisted in PVC. The aim of these trials was that ants could get familiarized both with the experimental set-up in which they were subsequently tested and with the manipulation they were subjected to during the test. The first familiarization trial started by randomly picking an ant in the foraging area of one of the experimental groups and by marking it individually with a spot of paint on the thorax. The ant was then transported on a toothpick that was inserted vertically in a tiny hole pierced at the centre of the empty channel. Once the ant had discovered one of the food sources, it was removed from the channel and transported back to the foraging area. In the first familiarization trial two droplets of 5 µl of 1 M sucrose solution were placed at the end of each arm of the channel. In the second familiarization trial only one food source was placed in the channel, on the arm opposite that where the ant had found the food source in the first familiarization trial. This allowed us to make sure that the ants explored the whole set-up before being submitted to the spontaneous preference test in the absence of food source.

In the test trial no food sources were placed in the channel and each half of the channel was covered by a layer of sand of different coarseness. All six possible combinations of the four types of sand coarseness obtained by the sieving process (400–560, 710–850, 1000–1120 and 1250–1400 μm) were tested. We considered that an ant had made a choice when it had crossed an unmarked line 3 cm from either side of the toothpick inserted in the centre of the channel. After the completion of the test the ant was placed apart from its experimental group to prevent being reused in another experiment. The sand was changed between each test trial run with successive ants. Twenty-six replicates of the experiment were achieved for each pair of sand coarseness with roughly the same number of individuals taken from three experimental groups (A, B and C).

2.3.2.2. Statistical analysis. To test whether ants express a spontaneous preference for one type of sand coarseness, we used a binomial test with a Bonferroni correction applied to the six combinations of sand coarseness tested. The null hypothesis was that ants should choose either side of the channel with equal probability if they have no spontaneous preference.

2.3.3. Experiment 3: tactile learning

In this last experiment we tested whether ants are able to learn the location of a food reward by using substrate coarseness as a cue. We used a positive reinforcement differential conditioning procedure (see Dupuy et al., 2006; Kleineidam et al., 2007 for other examples of the use of this procedure in ants) in which ants were given the choice between two substrates of different coarseness, with only one substrate being systematically reinforced by the presence of a food reward.

2.3.3.1. Experimental procedure. The same channel as in Experiment 2 was used in this experiment. As in Experiment 2, all tests were performed under red light.

Each ant ran through a series of trials consisting in two familiarization trials with the experimental set-up, 20 conditioning trials, and two test trials placed, respectively, between the 16th and 17th conditioning trial and after the last conditioning trial.

In the two familiarization trials ants were placed individually at the centre of the empty channel and were allowed to explore it until finding a droplet of 1 M sucrose solution. As in Experiment 2, one droplet was placed at the end of each arm of the channel in the first familiarization test, whereas in the second familiarization trial the food source was placed on the arm opposite that where the ant had found the food source in the first familiarization trial.

After these familiarization trials, ants were admitted again in the channel for the first conditioning trial. In the conditioning trials each arm of the channel was covered with a sand of different coarseness, either the finest (400–560 μm) or the coarsest (1250–1400 μm) sand ob-

tained by the sieving process. Two treatments were run, with a different sand coarseness reinforced with a food reward (a 5 μl droplet of 1 M sucrose solution) in each treatment. We considered that an ant had made a choice when it had crossed an unmarked line 3 cm from either side of the toothpick inserted in the centre of the channel. If the ant made its first choice towards the reinforced side, we closed the access to the other side until it found the food reward. On the other hand, if the ant chose the side without reinforcement, we let it explore the channel until it found the food reward in the other arm.

Each ant was trained individually along 20 conditioning trials with the same pair of sand coarseness. Because we tested freely foraging ants the intervals between successive training trials were not constant: the average value of the inter-trial interval for the two treatments was 4 min 30 s (mean \pm CI_{0.95}: 269.00 \pm 2.98 s, range: 185.00–589.00 s, $N = 800$) and there were no significant differences in the duration of inter-trial intervals between the two experimental groups. The sand was changed and the position of the sand coarseness pseudo-randomized between trials (over the 20 conditioning trials, the same sand coarseness was placed 10 times on the left arm and 10 times on the right arm). As in Experiment 1, ants were transported back to the foraging area of their nest after each conditioning trial, where they could exchange food with their nestmates. For each conditioning trial, we recorded the first choice of the ant and the time elapsed between their admission in the channel and the discovery of the food reward. After the 16th and the 20th conditioning trials, the ants were submitted to a test trial in which no reinforcement was placed in the channel. The ant's first choice was noted. The position of the sand coarseness was inverted between the two test trials.

In total, 40 ants belonging to three experimental groups were trained. In the first treatment, 20 ants were trained with the food reward associated to the finest sand (400–560 μm) and in the second treatment 20 ants were trained with the food reward associated with the coarsest sand (1250–1400 μm).

After the completion of the second test trial, ants were removed from their experimental group to prevent being retested.

2.3.3.2. Statistical analysis. Conditioning trials were analysed by blocks of four. For each block we computed the proportion of correct choices of each ant and averaged over all ants in the treatment group. To test whether the performance of the ants changed over successive trials and whether it differed according to the sand coarseness associated with the food reward (400–560 or 1250–1400 μm), we used a repeated measure analysis of variance with block as the first (repeated) factor and treatment as the second fixed factor. To comply with the assumption of normality and homoscedasticity the proportion of correct choices in each block was transformed by an arcsinus square-root transformation (Zar, 1996). We used

the Student *t*-test for independent samples with a standard Bonferroni correction to compare the proportions of correct choices observed in each block between the two treatments. To test whether there was any change over the four blocks of trials in the mean time to reach the food source and whether this change depended on the sand coarseness associated with the food reward, we used a repeated measure analysis of variance with block as the first (repeated measure) factor and treatment as the second fixed factor. We used a paired Student *t*-test followed by a standard Bonferroni correction to compare the performance of the ants between blocks.

The significance of the choice between the two sand coarseness in the two test trials was tested by a one-tailed binomial test. The null hypothesis was that after 16 or 20 conditioning trials ants should prefer the reinforced coarseness. The results of the two tests for each treatment were compared with a one-tailed McNemar change test (Siegel and Castellan, 1988). The null hypothesis was that more ants should change to choose the reinforced coarseness in the second test.

The assumptions of normality and homoscedasticity were tested with a Shapiro–Wilk test and a Levene's test, respectively. All analyses were performed with the statistical software SPSS for Windows, version 12.0 (SPSS inc., <http://www.spss.com>). All data in the text are given as mean \pm CI_{0.95}.

3. Results

3.1. Experiment 1: locomotory behaviour (Fig. 1)

The locomotory behaviour of the ants was significantly affected by the sand coarseness (MANOVA, sand coarseness effect; $F_{3,38} = 6.290$; $p = 0.001$) and differed among experimental groups (MANOVA: experimental group effect, $F_{12,120} = 3.488$; $p < 0.001$). Most importantly, there was no significant interaction between the two factors

(MANOVA: $F_{12,120} = 1.257$; $p = 0.253$), i.e. the locomotory behaviour of the ants was affected in the same manner by the substrate coarseness in the five experimental groups.

Two of the three variables used to characterize the ant locomotory behaviour were significantly affected by the sand coarseness: ants moved faster (linear speed: 2.63 ± 0.20 and 2.21 ± 0.17 cm/s, for the fine and coarse sand, respectively, one-way ANOVA: $F_{1,40} = 16.53$, $p < 0.001$) and their trajectories were slightly more sinuous (coefficient of directionality: 0.67 ± 0.03 and 0.71 ± 0.02 , one-way ANOVA: $F_{1,40} = 4.38$, $0.05 > p > 0.01$) on fine than on coarse sand. As for the value of the mean free path, it was not significantly affected by substrate coarseness (0.273 ± 0.026 and 0.245 ± 0.026 cm, for the fine and coarse sand, respectively, one-way ANOVA: $F_{1,40} = 3.29$, $p = 0.077$). The fact that the mean free path was not significantly different between the two sands and that the coefficient of directionality was greater on the coarse than on the fine sand indicates that the turning rate (amount of turn per unit distance) was greater for ants moving on the fine sand.

3.2. Experiment 2: spontaneous preference

Whatever the pair of sand coarseness tested, ants chose equally either sand coarseness during the test trial (none of the probability associated to the binomial test was less than the table-wide significance threshold of $0.05/6 = 0.008$). Thus, we conclude that ants did not express any spontaneous preference when faced with a choice between two sands of different coarseness.

3.3. Experiment 3: tactile learning

3.3.1. Conditioning trials

There was no significant improvement in the performance of the ants over the five blocks of conditioning trials (Fig. 2: ANOVA, block effect: $F_{4,152} = 0.640$; $p = 0.634$)

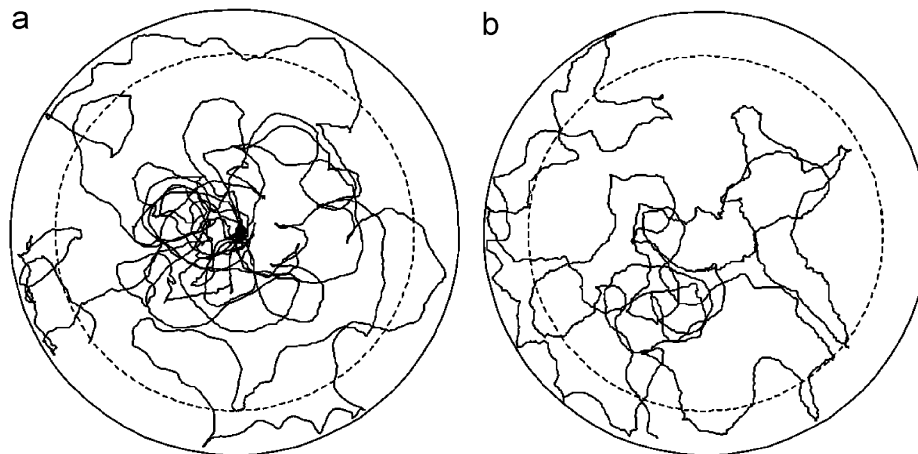


Fig. 1. Example of trajectory observed in Experiment 1 for ants walking on (a) fine sand or (b) coarse sand. The trajectories started at the centre of the area. The diameter of the area is 20 cm. To avoid edge effects, only the parts of the trajectories performed in the central zone (diameter: 18 cm) delimited by the dashed line were analysed.

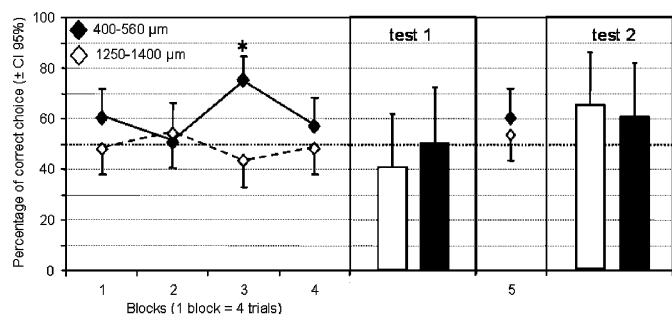


Fig. 2. Performance of the ants in the tactile learning experiment (Experiment 3). The percentage of correct choice made by the ants is shown for the 5 blocks of conditioning trials (in presence of food reinforcement) and for the two test trials run after the 16th (test 1) and 20th (test 2) conditioning trial (in absence of food reinforcement). One block corresponds to 4 conditioning trials. One group of ants was reinforced on fine sand (400–450 µm, close symbols, continuous line and black bars) and the other on coarse sand (1250–1400 µm, open symbols, dashed line and white bars). The dotted line indicates the level of random choice. An asterisk indicates that the scores of the two groups for a given block are significantly different at the 1% level of significance (Student *t*-test with a standard Bonferroni correction). For the 5 blocks of conditioning trials the scores correspond to the mean percentage of correct choice ($\pm 95\%$ confidence interval) of each ant over a block. The bars for each test correspond to the percentage of ants making a correct choice ($\pm 95\%$ confidence interval). $N = 20$ ants for each experimental group.

and the choice of the ants in each block of conditioning trials did not differ from random. However, ants trained on fine sand made significantly more correct choice than those trained on coarse sand (Fig. 2: ANOVA, treatment effect: $F_{1,38} = 11.912$; $p = 0.001$). The percentage of correct choices between the two treatments differed significantly only for the third block ($t = 3.117$; $p = 0.003$) with ants trained on the fine sand making more correct choices than those trained on the coarse sand.

There was no significant difference between treatments in the time to reach the sucrose reward (Fig. 3: ANOVA, treatment effect $F_{1,38} = 0.218$; $p = 0.644$). The time decreased significantly between the second and the third block of conditioning trials and increased again in the fifth block (Fig. 3: ANOVA, block effect: $F_{4,152} = 7.606$; $p = 0.009$).

3.3.2. Test trials

In both treatments ants did not choose the sand associated with the food reward when they were tested after the 16th (test 1) and after the 20th conditioning trial (test 2). Their choice did not differ from random in both tests (Fig. 2). The choice of the ants did not improve between the first and second test in both treatments (one-tailed McMemar change test: $\chi^2 = 0.083$, $p = 0.387$ and $\chi^2 = 1.230$, $p = 0.133$ for ants reinforced on the fine and the coarse sand, respectively).

4. Discussion

As expected, the locomotory behaviour of the ants was significantly affected by the coarseness of the substrate on

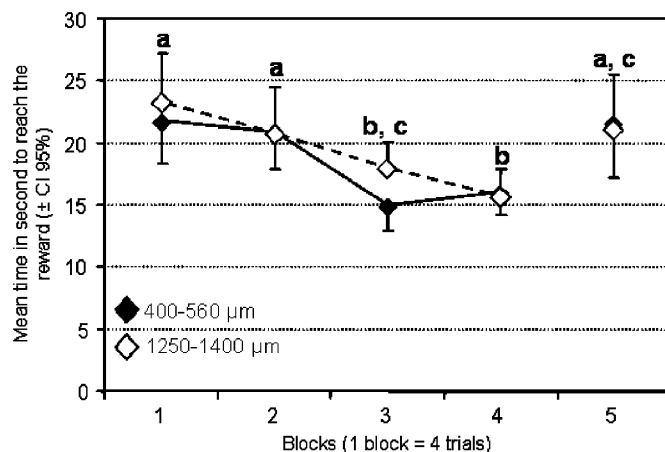


Fig. 3. Mean time $\pm 95\%$ confidence interval to reach the food reinforcement as a function of the 5 blocks of conditioning trials in the tactile learning experiment (Experiment 3). One block corresponds to 4 conditioning trials. One group of ants was reinforced on fine sand (400–450 µm, close symbols and continuous line) and the other on coarse sand (1250–1400 µm, open symbols and dashed lines). There was no significant difference in the time to reach the food source between the two groups. The symbols bearing the same letters indicate that the blocks were not significantly different at the 5% level of significance (Student paired *t*-test, followed by a Bonferroni correction). $N = 20$ ants for each experimental group.

which they were moving. Ants moved significantly faster and their trajectories were slightly more sinuous on the fine than on the coarse sand.

Ants walking on rough terrain moved more slowly probably because they had to reduce their stride length and stride frequency in order to keep their stability over the unevenness of the ground (Zollikofer, 1994b). Such a reduction in speed has been observed in other ant species. For example, workers of the Australian ant *Rhytidoponera aurata* (Nielsen, 2001) walking on a mowed lawn with very dense grass plants or those of the seed harvesting ant *Pogonomyrmex occidentalis* moving in environments with dense vegetation structure (Crist and Wiens, 1994) decrease their locomotory rate compared to those walking on bare soil. The locomotory rate of leaf-cutting ants walking on a layer of leaf litter (Rockwood and Hubbell, 1987) or that of seed-harvesting ants travelling along vegetated routes (Fewell, 1988) is also reduced compared to those walking on cleared routes. Note, however, that the value found for the speed of movement of an insect is highly dependent on the measurement method that is used. The actual locomotory rate of the ants on coarser substrates would be due to increase if one were to take into account not only the horizontal component of the trajectory of their centre of mass but also its vertical component. Coarser substrates have higher fractal dimension and the distance actually covered by an animal between two reference points can increase considerably with the fractal dimension of the surface on which they walk (Weiss and Murphy, 1988).

The fact that the turning rate was higher on the fine than on the coarse sand is more puzzling. In fact, according to

the results found by Zollikofer (1994a) in the ant *C. bombycina* a reduction in the speed of movement should be accompanied by an increase in the degree of path curvature. One hypothesis to explain our results is that *L. niger* workers exploring a new area with an even surface would spontaneously run in fairly convoluted trajectories. If this is so, bigger sand grains could constrain the insect's manoeuvrability and reduce the amplitude of their directional changes (Jindrich and Full, 1999). A fine kinematic analysis of the ant stepping pattern on the two types of sand would be required to investigate this hypothesis.

We found that ants did not express any significant preference for a particular coarseness when they had to choose between two sands of different coarseness. Therefore, either *L. niger* workers are not able to discriminate between the sand coarseness presented, or it has no ecological significance for them, i.e. there is no link between the location of the food sources they collect in their natural environment and the substrates presented.

We found little evidence of the effect of experience on substrate coarseness discrimination. When ants were systematically rewarded on one type of substrate coarseness, they did not significantly increase their choice for this coarseness over the 20 successive conditioning trials they achieved. Moreover, their choice remained random when they were tested in the absence of food reward after the 16th and 20th conditioning trials. Overall however, ants trained on fine sand made significantly more correct choice than those trained on coarse sand. Taking a learning criterion of four successive correct choices, which has a probability of $0.5^4 = 0.06$ to occur by chance alone, we found that 85% of the ants trained on fine sand and 40% of those trained on coarse sand reached the criterion in less than 20 conditioning trials. Moreover, the mean number of conditioning trials to reach the criterion was higher, although not significantly so, for the ants trained on coarse sand than for those trained on fine sand (12.50 ± 3.33 and 9.29 ± 2.25 , respectively, Student *t*-test: $t = 1.573$, $p = 0.129$). The dissymmetry between the two substrate contingency groups suggests that coarse sand may act as a negative reinforcement and facilitate the association of the food reward with the fine sand. This could occur if walking on coarse sand is energetically more costly than walking on fine sand. Since ants walking on coarse sand moved more slowly than those walking on fine sand (see Experiment 1), one could be tempted to say that they expended more energy per unit distance. However, the cost of transport, i.e. the amount of energy required to move one unit of body mass by one unit distance, is known to be independent of the speed of movement in ants (see e.g. Lighton and Duncan, 2002; Lipp et al., 2005). Therefore, the difference in the speed of movement alone cannot generate a difference in the energetic cost of running between the two substrates. Instead, there may be an additional cost of running on the coarse substrate if there is a postural cost associated to it, i.e. if ants have to expend energy to maintain their balance and stabilize their centre

of gravity when walking over the irregularities of the coarse substrate (Schmidt-Nielsen, 1972; Duncan, 1999). This hypothesis would deserve to be tested through accurate measures of the metabolic rate of the ants while running on the two types of substrates.

Much better results on the use of the physical properties of the substrate as a means to orientate in the environment were obtained by Seidl and Wehner (2006) in the desert ant *C. fortis*. In their experiment however, ants were tested in a natural foraging context after having completed about 30 training trials, whereas they achieved only 20 in our experiment. In ants, as in other insects (Papaj and Lewis, 1993), both the context of the experiment (nature/laboratory) and the ecological significance of the stimulus being used are important in forming an association between a stimulus and a reinforcer. For example, tactile stimuli may have more biological relevance in honeybees than in ants. Tactile stimuli are largely solicited in several behavioural activities in the darkness of the hive. Tactile sense is involved in particular to control the thickness of the cell walls (Kevan, 1987). Moreover, honeybees can use the fine-scale physical texture of petals to discriminate between flowers of different species (Kevan and Lane, 1985). It is therefore not surprising that free-flying honeybees are able to discriminate tactile cues of different grain size (abrasive paper) after only five conditioning trials (Erber et al., 1998). Our failure to condition ants to a tactile stimulus may also be explained by the fact that ants are generally difficult to condition in the laboratory. With a comparable differential conditioning procedure, *Camponotus* ants require more than 20 training trials before being able to discriminate between odours (Dupuy et al., 2006), a stimulus that has probably more biological significance for ants than substrate coarseness. Therefore, 24 training trials as in our experiment may not suffice to develop a learned coarseness preference.

The poor learning performance of the ants in our experiment could also be due to the experimental set-up and conditioning procedure we used. A differential conditioning procedure works better when there is a cost to respond to the unreinforced stimulus. In our experiments, there was a cost for ants to first choose the unreinforced substrate coarseness because in that case they reached the food source less rapidly (mean time to reach the food source: 28.51 ± 1.88 s vs. 12.29 ± 1.55 s when ants made an incorrect vs. a correct choice, respectively; Student paired *t*-test: $t = 18.71$, $df = 39$, $p < 0.001$). This was due to the fact that the ants that first chose the reinforced side of the channel were immediately prevented by a physical barrier from accessing the other (unreinforced) side of the channel. Our conditioning procedure may have been more effective with a higher cost associated in choosing the unreinforced side of the channel. The use of a longer channel (Seidl and Wehner, 2006) or a conditioning procedure with a negative reinforcement on the unrewarded side of the channel (e.g. a droplet of quinine solution as in Dupuy et al. (2006)) may have yielded

different results. On the other hand, the absence of learning was not due to a lack of motivation. Indeed, the fact that there was a significant decrease in the time to reach the food source over the first 16 conditioning trials (Fig. 3) shows that ants were motivated to search for and feed on the food source. Note also that this motivation was relatively fragile since one trial without reinforcement (test 1) was sufficient to raise the time to reach the food source to the level measured in the first block of conditioning trials.

Finally, the last explanation for the poor learning performance we observed could be that *L. niger* workers lack the sensory equipment to discriminate between the coarseness presented. There are several ways one could think of for ants to measure the coarseness of the substrate on which they walk. First, ants possess mechanoreceptors on their antennae (Hölldobler and Wilson, 1990) and they could use these latter to sense and assess the coarseness of the substrate. However, in ants as in honeybees (Erber et al., 1998), the antennae are probably used to measure the coarseness of vertical rather than horizontal surfaces. Second, ants could measure surface coarseness by tarsal contact. This is unlikely however since the grain size of the sands we used was approximately half the size of the ants for the coarser sand and the fourth of their size for the finest sand. This is much too large for sand coarseness to be discriminated on the basis of the mechanoreceptors located on the tactile hairs of the workers' tarsi (Freeland et al., 1982). And third, one way ants could measure the coarseness of a substrate could be through the *campaniform sensilla* located within their cuticle. These sensilla consist of sensory neurons whose dendrite inserts into a cap at the surface of the cuticle and that are sensitive to the forces applied on the exoskeleton while the insect is walking (Zill et al., 2004). Some of these sensilla are located at the body joints (head, alitrunk, gaster) and they can sense the changes in the body postures of the insect while it is walking on complex terrain (Ritzmann et al., 2004). In addition, a lot of insects, including ants (Markl, 1962, 1963; Wittlinger et al., 2007), are equipped with sensitive hair plates at the body joints through which they can determine the relative position of their body parts. It is possible however that, despite the change in locomotory behaviour we observed for ants walking on different substrate coarseness (Experiment 1), the difference in grain size of the sands we used was not sufficient to significantly modify the forces applied on the exoskeleton or to induce a change in body postures detectable by the hair plates. A kinematic analysis of the ants' body posture while walking on the different type of sands would be necessary to test this hypothesis.

In summary, our study shows that the ants are not able to discriminate different substrates on the basis of their mechanical properties, although their locomotory behaviour is significantly affected by these properties. At least in the species of ants using trail pheromone to recruit to abundant food sources, substrate coarseness may in fact

have more consequences at the collective than at the individual level. Substrate coarseness can indeed affect the trail-laying behaviour of recruiting workers or the accessibility of the pheromone to the chemical receptors of recruited ants. The physical properties of a substrate can also interact with the chemical properties of the trail pheromone, in particular its evaporation rate (Jeanson et al., 2003). It could thus strongly influence the dynamics of recruitment to a food source and, ultimately, the choice of a path along which a trail is established. For example, Detrain et al. (2001) have shown that when *L. niger* workers were given the choice on a diamond-shaped bridge between a branch covered by a light-weight paper and one covered by a heavy-weight paper, they eventually selected the branch covered by the light-weight paper in the majority of the experiments. This shows that the choice of a substrate on which to establish a trail depends more on the interaction between the physical properties of the substrate and the chemical properties of the pheromone than on the ants' individual behaviour or their capacities to discriminate between different substrates.

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