SPATIO-TEMPORAL LEARNING BY THE ANT *ECTATOMMA RUIDUM*

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Summary

We tested, under field and laboratory conditions, whether the neotropical ant *Ectatomma ruidum* Roger can learn several associations between temporal and spatial changes in the daily pattern of food availability. Honey was shuffled between two or three feeding sites following a fixed daily schedule. Foragers learnt to associate particular sites with the specific times at which food was available, individually marked ants being observed on the correct sites at the correct times. Some ants anticipated the time of food delivery by approximately 30 min, and it was not necessary for them to be rewarded at the first stage of the sequence of food collection to continue their search for honey according to the correct schedule of reward. Ants also followed the same schedule when no honey was supplied at each stage of the sequence, and they stayed at the expected unrewarded site for a period equivalent to the reward period of the corresponding training phase, indicating that they had learnt when and for how long the food was available. Thus, ants rely on their spatio-temporal memory rather than on local cues coming from the honey source to guide them.

Key words: spatio-temporal learning, cognition, foraging activity, ponerine ant, *Ectatomma ruidum*.

Introduction

During evolution, most animal species have developed navigational abilities that allow them to look for remote familiar places more efficiently than by moving in a random way. Some vertebrate and invertebrate species can also encode the time at which significant biological events occur. Such control of temporal information allows the individual to adapt to the time structure of its environment by estimating the time of occurrence and the duration of regular events (Gallistel, 1990). This time control has been well documented in social insects (von Frisch, 1965; Fourcassié et al., 1999). Some species can also learn to regulate, at the individual level, both spatial and temporal information in a more complex time–place integration process. It has been shown that mammals, fishes and birds can learn specifically where and when to look for food (Rijnsdorp et al., 1981; Terborgh, 1983; Biebach et al., 1989; Reebs, 1996; Wilkie et al., 1996).

The goal of the present study was to determine whether ants can learn to forage in different places at different times of the day, each place being associated with a specific time. Our observations were conducted on the neotropical ponerine ant *Ectatomma ruidum* Roger, in which foragers fall into five behavioural categories: sugar collectors, hunters, unspecialised intermediates, patrollers and nest-maintenance workers (Schatz et al., 1995b, 1996). Two sets of experimental evidence indicate that *E. ruidum* is able to perform either temporal or spatial learning. First, individual workers can learn to search

for honey at one specific time of day despite a natural triphasic circadian rhythm of feeding activity (Schatz et al., 1993). Such temporal learning is surely of great adaptive value for *E. ruidum* because, in its tropical environment, carbohydrate food sources are mainly provided at restricted times of day by extra-floral nectaries (Weber, 1946; Lachaud, 1990; Passera et al., 1994). Second, workers of this species forage individually both in the field (Lachaud et al., 1984) and in the laboratory, where an individual will frequent one given carbohydrate food source out of several possible sources (Schatz et al., 1993). In this case, the preferred food site is localised through visuo-spatial learning, without the help of chemical information (Schatz et al., 1995b).

One aim of the present study was to test whether the learning of a time–place pattern, as seen in the laboratory (Schatz et al., 1994), persisted in the natural habitat even outside the periods of the natural triphasic activity rhythm of this species. The second aim was to test whether ants could learn several time–place patterns and display them in the absence of food. Our earlier experiments did not specifically investigate possible interactions between the retention of time–place patterns and responses to local cues (Franklin, 1994; Schatz et al., 1994). One simple strategy would be for the insect to respond only to local cues emanating from sugar. The ant could search until it located the rewarded site, either randomly or directed by the odour plumes from the food source, and then

remain there until the food source was extinguished. Social insects could also exploit an ephemeral source at the colony level by using a recruitment strategy, as in the case of some nectar-feeding ant species (Herrera et al., 1984). Another strategy is to use spatial and temporal memory and to learn a specific time–place association for each appointment (Biebach et al., 1994). A representation of several independent place-and-time patterns implies that the animal can learn to visit different particular places at the correct specific times of day irrespective of the reward at one or the next stage of the daily schedule of food availability. Here, we present experimental evidence that such a joint representation of spatial and temporal information exists in *E. ruidum* both in natural and in laboratory conditions.

Materials and methods

Description of the colonies

Ants of the species *Ectatomma ruidum* Roger were either observed in their natural habitat in the neotropical region of Chiapas, Tapachula, Mexico, or collected and reared in the laboratory in Toulouse, France. Both in the field and in the laboratory, the natural triphasic rhythm of foraging activity occurs at 09:00–10:00 h, 11:00–12:00 h and 15:30–16:30 h (Lachaud, 1990; Schatz et al., 1993; Passera et al., 1994).

In the field, two colonies (A and B) with 99 and 32 workers, respectively, were used. The size of the colonies was determined after complete excavation at the end of the experiments. In the laboratory, experiments were conducted with three queen-right colonies (colonies C, D and E) with 35, 72 and 112 workers, respectively. During preliminary observations, all workers performing outside activity were marked individually with enamel paint on their thorax and gaster. In all, 38 and 16 workers were marked in the two field colonies (A and B), respectively, and 17, 30 and 42 workers were marked in the three laboratory colonies (C, D and E, respectively).

Experimental apparatus

In the field, two experimental food sites (S1 and S2) were placed in the foraging area of each colony (A and B) to allow direct observation of both sites by a single experimenter. The two sites offering honey were covered by a transparent round box (diameter 10 cm), pierced with small holes at the bottom to allow the passage of ants but to prevent bees from entering. The sites were oriented at 90° to each other and placed at a distance of 50 cm from the nest entrance.

In the laboratory, each colony (C, D and E) was maintained in a plaster nest under the controlled conditions of the experimental room (temperature 25 ± 1 °C; relative humidity 60 ± 5 %; 12 h:12 h L:D photoperiod starting at 08:00 h). Each nest was connected *via* plastic tubes to a box (diameter 10 cm) containing frozen crickets as a source of protein and to the centre of an arena (diameter 30 cm) from which foragers had access through plastic tubes to three different honey sites placed 120° apart at the periphery of the arena (Fig. 1). The
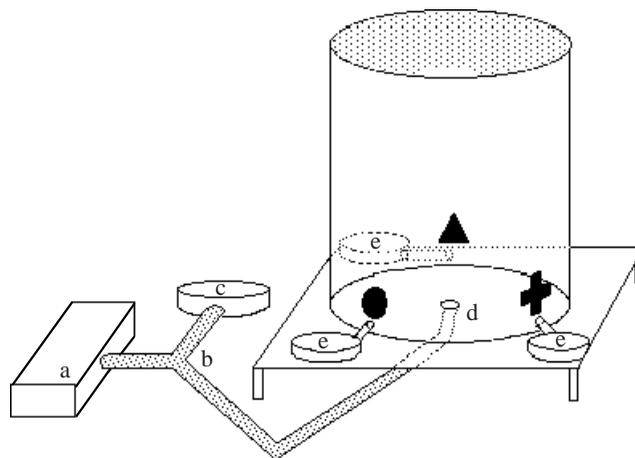

Fig. 1. Experimental arrangement. Colonies of the ant *Ectatomma ruidum* Roger were reared in plaster nests. The nest (a) was connected *via* plastic tubes (b) to a box containing crickets as a source of protein (c) and to the centre of an arena (d) (diameter 30 cm) from which foragers had access to carbohydrate food sources. To find honey without direct guidance cues from the food source, workers had to locate the entrance to one of three tubes placed 120° apart at the periphery of the arena. Each tube was linked to a box (e) containing a dish filled with honey. The location of the entrance of each tube was indicated by a specific visual landmark (a triangle, a cross or a circle) placed above the tube entrance on the inner wall of the arena.

location of each honey site was indicated by a specific visual landmark (a triangle, a cross or a circle) placed above the entrance to each tube on the inner wall of the arena. This arrangement made it impossible for an ant situated in the arena to see whether another ant was present at one of the three honey sites; its decision to enter a tube was thus not influenced by the presence or absence of nestmates. The three colonies were familiarised with the experimental arrangement for 2.5 months, during which ants were free to visit any site at any time and to collect honey. When required, crickets were provided after 17:30 h to avoid any disruption to the training procedure.

Training procedure

In the field

Honey was available in the first site (S1) from 09:10 to 10:10 h and in the second site (S2) from 12:10 to 13:10 h, i.e. outside the periods of the second (11:00–12:00 h) and third (15:30–16:30 h) peaks of the spontaneous activity rhythm of foraging *E. ruidum* (Lachaud, 1990; Schatz et al., 1993; Passera et al., 1994). Food was provided on 11 successive days, with D0 indicating the first day of reward presentation. The dish containing honey was removed after each observation period and replaced with a clean empty one. Spatio-temporal learning was tested on day 12 (D12) without honey in the dishes. Honey was provided again during the two following days (D13 and D14), and spatio-temporal learning was tested a second time with empty dishes on D15. The two feeding sites and the nest entrance were observed from the same position by

the same experimenter, and the individuals present at each site were counted every 5 min between 08:10 and 14:05 h.

In the laboratory

To increase the level of complexity of the learning task, honey was provided at three different sites during the day. Honey was available at the first site from 09:00 to 10:00 h, at the second from 11:00 to 12:00 h and at the third from 15:30 to 16:30 h. After each feeding period, the dish containing honey and the plastic tubes between the dishes and the arena were removed and replaced with clean empty ones. The three feeding sites were observed from the same position by the same experimenter, and the number of individuals present was counted every 15 min between 08:00 and 17:30 h. As expected from previous studies (Lachaud, 1985; Schatz et al., 1994), no mass recruitment was observed in the search for honey at the three feeding sites. Moreover, the number of ants present at a feeding site did not increase rapidly, but remained relatively stable when food was provided at the beginning of the reward period, confirming that recruitment did not occur in this situation. Thus, it is unlikely that odour trails are deposited by workers when they forage on small amounts of sugar under our laboratory conditions (Schatz et al., 1997). Nevertheless, since *E. ruidum* workers have been known to lay a chemical trail when searching for long-lasting sources of carbohydrate in the field (Pratt, 1989), the floor of the arena was cleaned with alcohol at the beginning of each of the 4 days of observation (D12, D15, D18 and D22) to eliminate any possible chemical directional information.

The following experiments were carried out. (1) To estimate the number of entries of ants (i.e. each time an ant entered into the feeding site) that occurred in the presence of honey following training as a control situation, spatio-temporal learning was tested on D12 and D15 after the 11 day training phase using the same reward schedule. (2) To test whether it was necessary for ants to be rewarded at the first stage of the sequence to remember the appropriate following locations and correct times of food availability, honey was provided on D18 only at the last two sites (at 11:00 h and 15:30 h). (3) A final test with no food reward was conducted on D22 to determine whether ants could rely on their spatio-temporal memory without local cues coming from the food source to guide them throughout the day. The normal training reward schedule was provided on the days between the tests.

Statistical analyses

For the field tests, we considered that a worker had performed a simple form of temporal learning if it visited one of the two sites during the corresponding reward period. We considered that a worker had performed spatio-temporal learning if it visited the two correct sites in the right order and at the right time. We defined three possible foraging zones: the two honey sites (S1 and S2), spatially defined by their covered boxes, and the rest of the foraging area (S3). The probability of entries into each site was defined as the number of entries into that site divided by the total number of entries into the three sites. We did not observe significant differences in the pattern of entries between colony A and colony B. For the larger colony (A), we counted 29 (S1), 25 (S2) and 31 (S3) entries, respectively, for the first test on D12, and 30 (S1), 29 (S2) and 28 (S3) entries for the second test on D15. Because there was no significant difference in the number of entries at the three sites between the two test days (χ^2=0.355; d.f.=2), the number of entries at each site was pooled. We then used the binomial test with probabilities of entry of 59/172 at S1 and 54/172 at S2 (associated with probabilities of no-entry of 113/172 and 118/172, respectively). Note that this proportion of approximately one-third is much greater than expected: the probability of entry into one of the two rewarded sites should actually be 3.3 % and not one-third given the surface ratio between a circle of diameter 10 cm (honey site) and a circle of diameter 55 cm (circle centred on the nest entrance and including the two honey sites).

For laboratory tests, we also distinguished between individuals that had learnt either two or three reward periods. This distinction was made only to determine the relative proportions of workers in these two categories, and statistical tests were performed on all individuals from the two categories. For each test, we pooled the number of entries of workers belonging to the three colonies. Statistical analysis was performed using a binomial test for each time check. As the three sites had the same theoretical probability of entry (one-third), we tested the number of entries at each site by making the assumption that one-third of entries would be at the rewarded site and the other two-thirds at the two unrewarded sites. For each time check, we determined the probability of obtaining the actual number of entries at each site. However, the binomial test could be used only if the number of entries was at least three; consequently, no test was performed for values below this. The times that the workers stayed in honey sites are given as means ± S.D.

Results

Spatio-temporal learning in the field

Analysis at colony level

In the field, the spatio-temporal availability of honey involves a local increase in the number of entries to the two rewarded sites (Fig. 2). We established several categories of workers in colony A according to the type of learning they performed and to the presence or absence of colour marks on them. Workers that had learnt the two spatio-temporal patterns contributed 30.9 % of the total number of the entries during the two tests. Some ants (9.1 % of entries) only learnt to enter one of the two honey sites at the right time. Some workers (2.9 % of entries) did not display any temporal learning. Of all entries, 57.1 % were performed by unmarked individuals, i.e. by workers from neighbouring colonies. Of the 42.9 % of entries of marked workers, 72.0 % were performed by ants that had learnt both time–place patterns, 21.3 % by individuals that had learnt one site and 6.7 % by individuals showing no learning. Fig. 2 shows the

heterogeneity of individuals entering the reward sites according to the category of learning performed and to the nest to which they were belonged.

Individuals classified as having learnt both sites visited them at the right times and during approximately the hour corresponding to the training period (Fig. 2). Although the time schedule of the second reward period was situated outside the usual peak of activity of this species, this site was visited just as precisely as the site of the first reward, which occurred within the usual morning peak of activity (Schatz et al., 1994). This underlines the high degree of flexibility of *E. ruidum*

workers in the temporal organisation of their foraging activities in natural conditions.

Analysis at the individual level

Fig. 3 shows the locations of 15 workers (12 individuals from colony A and three from colony B) at 10 min intervals during the second test day (D15). These records clearly show that the ants were present at the feeding sites corresponding to food availability during the learning phase. During the two test days without reward, 17 different individuals expressed the correct spatio-temporal schedule (14 from colony A and three
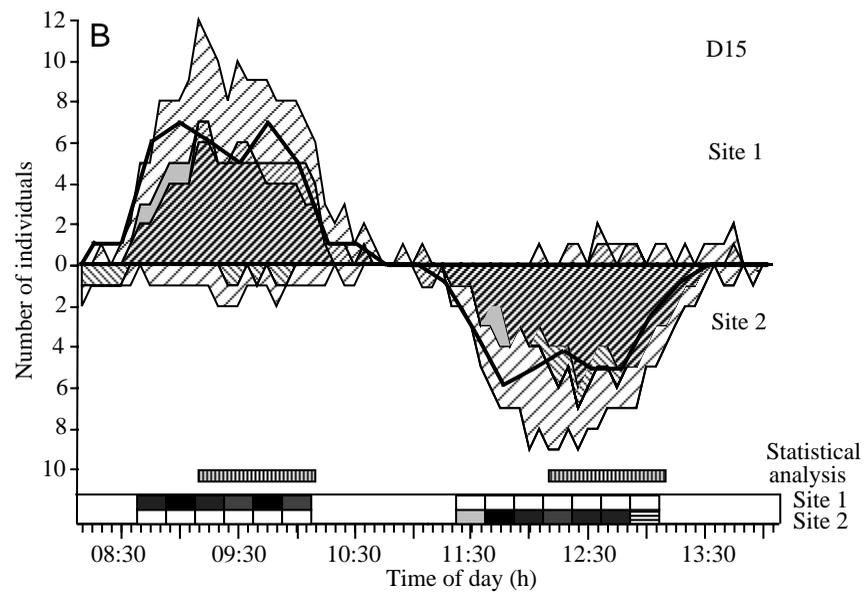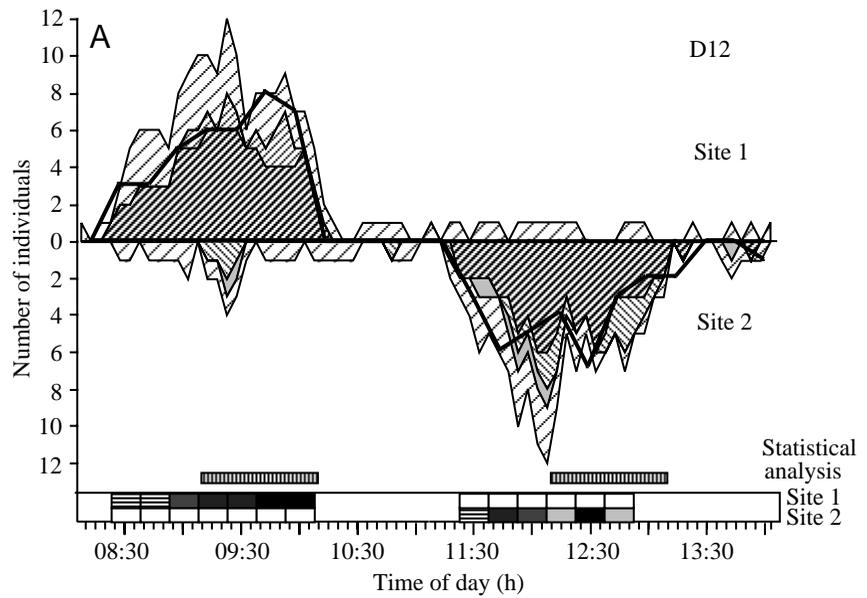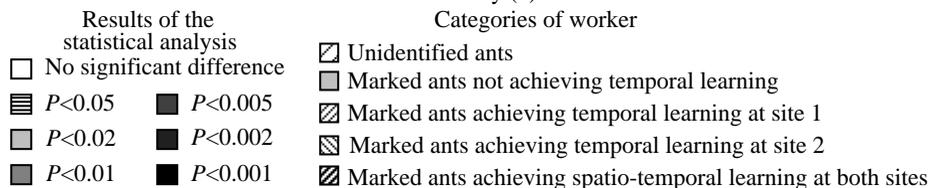

Fig. 2. Distribution of ants in the field at the two empty honey sites on days 12 (D12, A) and 15 (D15, B). The different hatched sectors correspond to the number of ants of the different categories present (recorded every 5 min), and the thick line corresponds to the number of entries of all workers that had achieved spatio-temporal learning (records are pooled every 15 min). On the basis of the values observed, we used the binomial test with probabilities of entry of 59/172 at S1 and 54/172 at S2 associated with probabilities of no-entry of 113/172 and 118/172, respectively. The vertically hatched bars below the figure indicate the period during which honey was supplied in site 1 (9:10–10:10 h) and site 2 (12:10–13:10 h) during training. The blocks at the bottom on the figure show the results of the statistical comparisons with different levels of significance coded by shading as shown.

Results of the statistical analysis

□ No significant difference	
▤ *P*<0.05	▨ *P*<0.005
▨ *P*<0.02	■ *P*<0.002
▨ *P*<0.01	■ *P*<0.001

Categories of worker

▨ Unidentified ants
▨ Marked ants not achieving temporal learning
▨ Marked ants achieving temporal learning at site 1
▨ Marked ants achieving temporal learning at site 2
▨ Marked ants achieving spatio-temporal learning at both sites
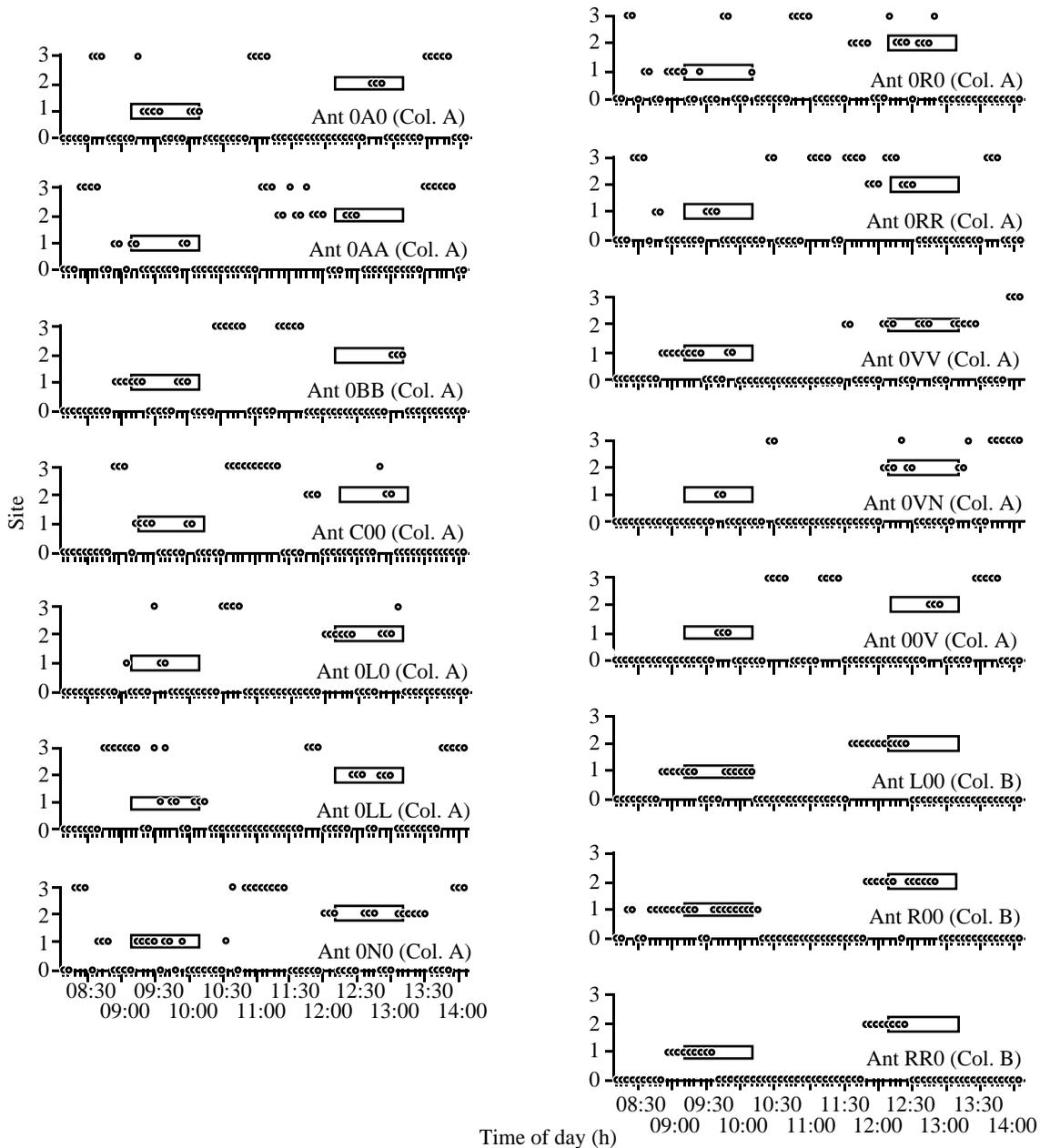
Fig. 3. Location of the 15 ants foraging on day 15 (site 0 corresponds to the nest, sites 1 and 2 to the two feeding sites and site 3 to the rest of the foraging area). Each point indicates a visit to a specific site as a function of time of day in 10 min blocks. Rectangles show times when honey was normally available at the feeding sites during training (but not during the tests). The code number indicated at the bottom right of each graph identifies each individual ant and colony.

from colony B) with eight individuals in common for the two tests. All individuals from colony A that had learnt the spatio-temporal patterns also made at least one entry into the rest of the foraging area (S3). That spatio-temporal learning allows accurate organisation of foraging at an individual level is clear because none of the 17 workers entered site 1 after 10:40 h or site 2 before 11:15 h.

Spatio-temporal learning in the laboratory
Control tests with reward: analysis at colony level

On D12 and D15, the entries of ants to the three foraging

sites were organised according to a spatio-temporal schedule. For each day of testing, the periods during which entries occurred were significantly different from a random distribution and corresponded to the reward periods (Fig. 4).

Control tests with reward: analysis at the individual level

Analysis at an individual level confirms that workers learnt to associate the three reward periods with the three reward sites. Among workers foraging at the honey sites on the two test days, we could distinguish four categories: (1) workers entering all three reward sites (N=45; 73.9% of the total
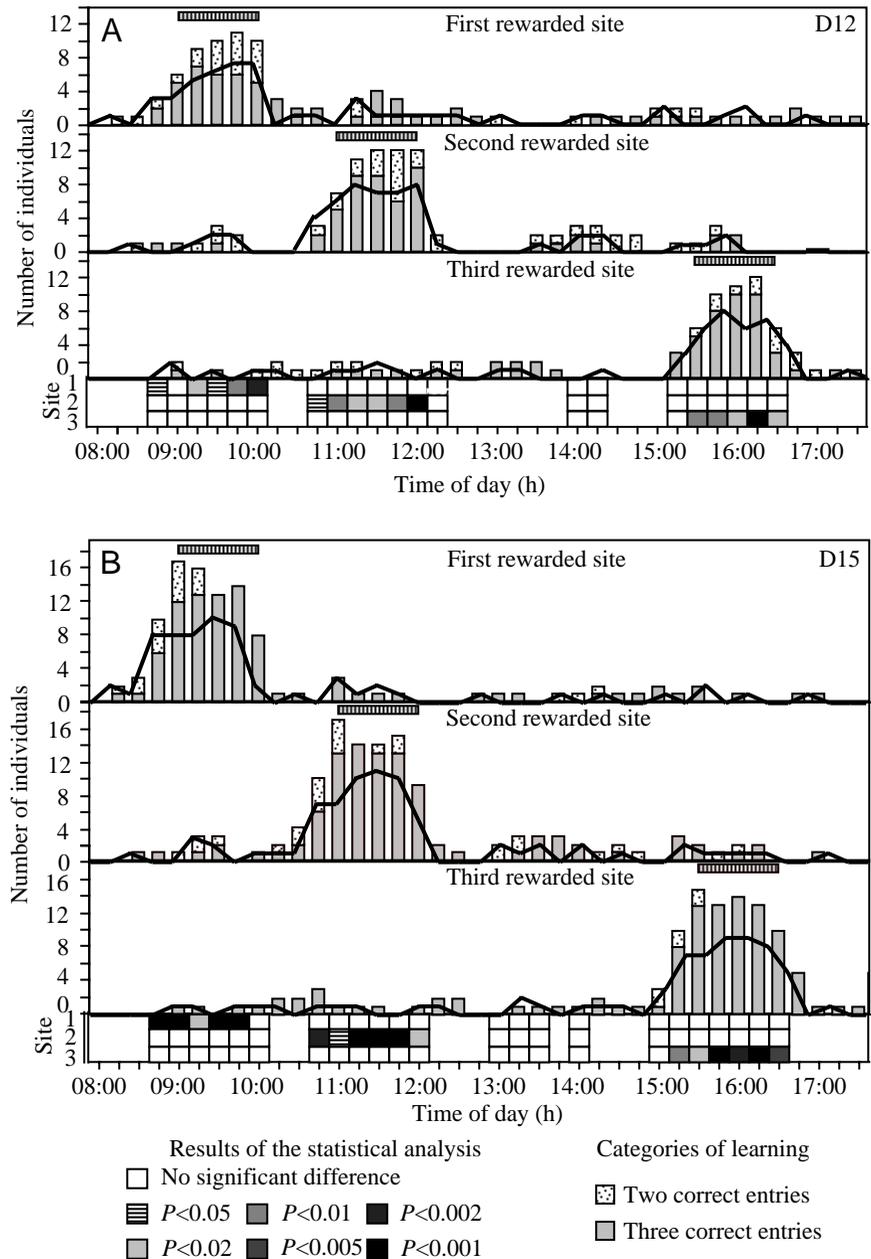
Fig. 4. Distribution of ants at the three sites in the presence of honey on days 12 (D12, A) and 15 (D15, B). The different hatched bars correspond to the number of ants of the different categories present, and the continuous line corresponds to the number of entries into each honey site of workers that had achieved spatio-temporal learning. As the three sites had the same theoretical probability of entries (one-third), we tested the number of entries at each site by making the assumption that one-third would be at the rewarded site and the other two-thirds at the two unrewarded sites. The vertically hatched bars below the figure indicate the period during which honey was supplied in the three sites during training and on the test days. The blocks at the bottom of each figure show the results of the statistical comparisons between the entries of workers that had achieved spatio-temporal learning and a random distribution, with different levels of significance coded by shading as shown.

number of entries for the two tests), (2) workers entering two reward sites (N=17; 18.1 % of total entries), (3) workers entering a single site (N=10; 5.7 % of total entries), and (4) workers exhibiting no temporal learning (N=6; 2.3 % of total entries) (Table 1).

A comparison of the numbers of individuals in the four learning categories between D12 and D15 indicates that individuals became better at the task with more training (Table 1). The number of individuals entering three reward sites increased between D12 and D15, while the numbers entering two or one reward sites or showing no learning decreased (Table 1). There was a significant difference in the distribution of individuals among the four behavioural categories (McNemar sign test: P<0.001). If we consider the four categories to indicate an improvement in learning as one

moves from no learning to the spatio-temporal learning of three reward periods, we can identify, among the 20 individuals in common for the two tests, 13 ants that improved their performance, six in which the performance was unchanged and one in which it became worse.

During the two test days in the presence of honey (D12 and D15), 18 individuals showed spatio-temporal learning at the three sites (four, six and eight individuals from colonies C, D and E, respectively), and 11 individuals learnt two time–place patterns (four, three and four individuals from colonies C, D and E, respectively). The majority of honey collectors show a preference for one honey site (Schatz et al., 1994, 1995a). However, in the present study, incorrect entries mostly occurred outside the reward periods. The foraging patterns of honey collectors were thus organised according to a pattern of

Table 1. *Number of workers and entries into the honey sites for four categories of worker defined according to the level of learning shown on days 12 (D12) and 15 (D15)*

Learning level category	Number of workers			Number of entries	Percentage of entries (*N*=299)
	Test 1 (D12)	Test 2 (D15)	Total		
Three time–place patterns	18	27	45	221	73.9
Two time–place patterns	11	6	17	54	18.1
Temporal (single site only)	7	3	10	17	5.7
No learning performance	4	2	6	7	2.3
Total	40	38	78	299	100

The percentage of entries is based on the total number of entries performed by each cateogry.

rewarded periods, when workers visited the rewarded sites, and unrewarded periods, when workers visited their preferred honey site.

Use of local cues versus spatio-temporal memory
Absence of the first reward

In spite of the absence of honey during the normally rewarded first period at the first site (Fig. 5), 22 out of 26 workers (seven, seven and eight individuals from colonies C, D and E, respectively) continued to enter all three sites at the appropriate times, with the remaining four ants entering two sites (two, zero and two, respectively). Workers entering the first site remained there for approximately 1 h corresponding to the training period. These results show that it was not necessary for the insects to be rewarded during the first test period, they still continued to search for honey according to the trained schedule of rewarded sites.

Tests without reward

In spite of the total absence of reward during D22, the entries of the workers to the three sites were significantly different from a random distribution, showing a peak according to the reward schedule during training (Fig. 6). In this test, 26 individuals entered three sites appropriately (eight, nine and nine individuals from colonies C, D and E, respectively), and four individuals entered two sites (one, zero and three, respectively) and stayed at each site for approximately 1 h.

Other characteristics of spatio-temporal learning
Anticipation

The existence of anticipation was examined by pooling the number of entries to a site before the predicted reward time within the same type of test (Table 2). There is clearly a 15 min anticipation period, in addition to which a 30 min anticipation period can be demonstrated (*P*<0.025) by pooling the data from all the experimental situations. From the field tests, the number of entries to a site also increased significantly 35 min before the reward period.

Duration of trips

The duration spent in a foraging site by an ant is another variable of interest regarding the influence of training on
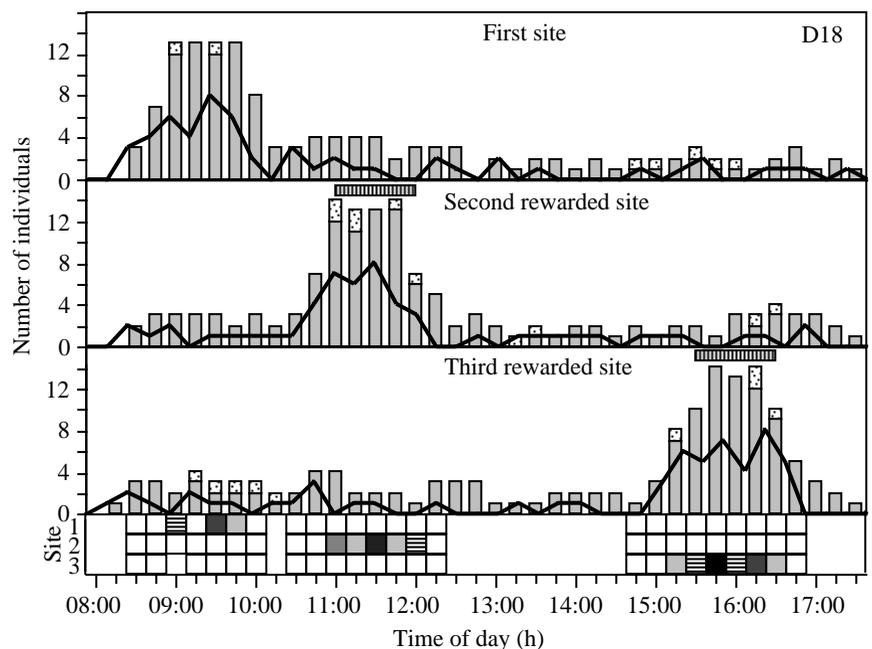

Fig. 5. Distribution of ants at the three sites on day 18 (D18). Honey was only supplied at sites 2 and 3 (vertical hatched bars) during the test day. During training, honey was also supplied at site 1 (between 09:00 and 10:00 h). Other details are as in Fig. 4.
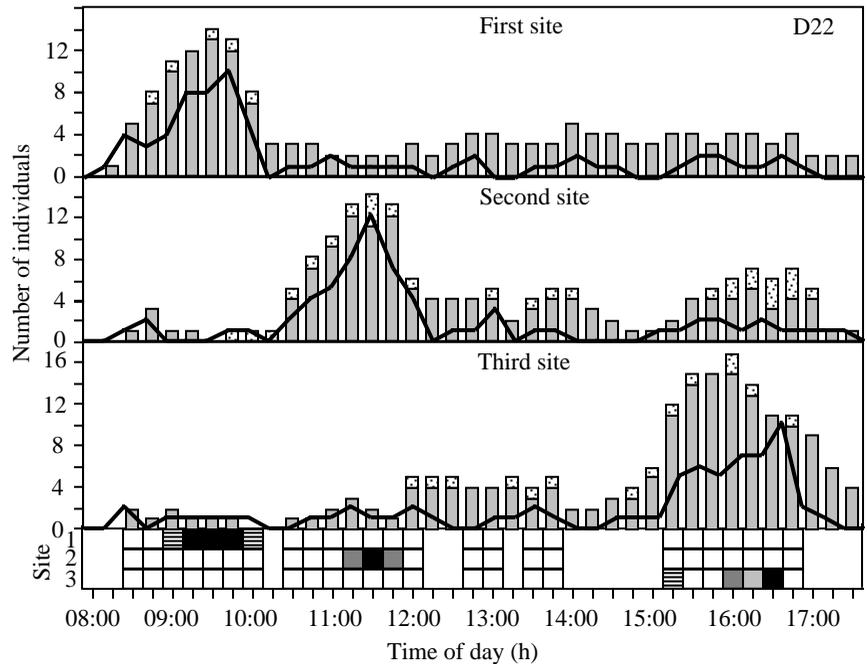
Fig. 6. Distribution of worker ants at the three honey sites during day 22 (D22). During training, honey was supplied at site 1 between 09:00 and 10:00 h, at site 2 between 11:00 and 12:00 h and at site 3 between 15:30 and 16:30 h. Other details are as in Fig. 4.

individual patterns of foraging. The mean duration of a trip during D15 in the laboratory (17.92±11.03 min, mean ± S.D., N=162) was significantly shorter than the duration of a trip during D12 (21.77±15.81 min, N=113; t=2.24; P<0.05), again indicating an improvement in learning between these two rewarded tests. Larger differences exist between the duration

of a trip on D15 test with reward and the durations measured on the D18 test without the first reward (32.52±20.65 min, N=134; t=7.36; P<0.001) and the D22 test without any reward (42.39±35.10 min, N=138; t=7.86; P<0.001), suggesting that the absence of the reward is associated with an increase in the duration of the trip.

Table 2. *Estimated duration of anticipation in laboratory and field tests*

	Duration before reward (min)	Test with reward	Test without reward at site 1	Test without reward	All tests
Laboratory	15	32/5***	14/8**	12/6**	58/19***
Laboratory	30	5/7	7/9	7/5*	19/21*
Laboratory	45	4/2	2/5	2/0	8/7
Field	5			7/0***	
Field	10			8/0***	
Field	15			8/0***	
Field	20			9/0***	
Field	25			6/0**	
Field	30			7/0***	
Field	35			6/0**	
Field	40			2/0	
Field	45			2/0	

Within each type of test, the numbers of entries before the correct time of the reward were pooled.

In each case, the number on the left indicates the number of entries into the rewarded site, the number on the right indicates the number of entries into unrewarded sites.

Asterisks indicate results of statistical comparisons between entry to rewarded *versus* unrewarded sites (binomial test): *P<0.05; **P<0.005; ***P<0.001.

Discussion

Vertebrates can associate different feeding places with different times of day. Several bird species, such as garden warblers *Sylvia borin* (Biebach et al., 1989, 1994; Krebs and Biebach, 1989), pigeons *Columbia livia* (Wilkie and Willson, 1992, 1995; Saksida and Wilkie, 1994; Wilkie et al., 1994), gulls *Larus* spp. (Wilkie et al., 1996, 1997), starlings *Sturnus vulgaris* (Wenger et al., 1991) and weavers *Ploceus bicolor* (Falk et al., 1992) can individually learn temporal and spatial associations in the pattern of food availability. In the same way, the ability to form time-and-place associations was recently reported in a cichlid fish (Reebs, 1996). Many studies have been devoted to the integration of temporal and spatial information in the behaviour of social insects (von Frisch, 1965; Menzel et al., 1998).

In all temporal learning experiments described previously in bees and ants, it appeared that spatio-temporal learning was implicit since these animals were able to associate different times of day with the spatial information that characterises the location of the food sources visited (for a review, see Fourcassié et al., 1999). However, in most cases, the location of the food sources remained unchanged throughout the day, whereas a rigorous demonstration of true spatio-temporal learning requires the location of the food sources to be changed at each period of reward (Biebach et al., 1989). It is clear that bees and ants can be trained to feed at a feeding site at the same

time every day (Beling, 1929; Wahl, 1932; von Frisch, 1965; Harrison and Breed, 1987), although this does not necessarily imply that they have learnt an association between different pairs of time–place patterns (Biebach et al., 1989; Fourcassié et al., 1999). Koltermann (1974) found that individual bees can be trained to feed at the same site at nine different times of day, indicating that bees have a temporal learning ability but not demonstrating a time–place learning ability such as would be shown by, for example, associating nine different times of day with nine different places.

Apart from a recent study by Menzel et al. (1998), the effect of recruitment on visiting bees is often not fully taken into consideration in experiments training bees to feed at different places at restricted times of day (Wahl, 1932; von Frisch, 1965). Indeed, Lindauer (cited in von Frisch, 1965) reported that bees can indicate both time and location information in their dance behaviour during recruitment of nestmates. Thus, as discussed by several authors (Medugorac and Lindauer, 1967; Moore et al., 1989; Frisch and Koeniger, 1994), the few individuals that visited each site at the right time could have been recruited by a small group of nestmates that had specialised in one reward period only, without actually having learnt an association between several time-and-place patterns.

Our work indicates that non-recruited individual ants of *Ectatomma ruidum* learn the association between different sites and different times, even outside the period of their spontaneous rhythm of foraging activity, and consequently that the performance observed depends merely on a combination of time control and spatial learning (Schatz et al., 1994, 1995a). Despite individual variability in the choice of time and/or site during the day, it is clear that, in the absence of any cues indicating honey availability, each forager learns to visit different places to feed at different times of the day. In addition, our data not only indicate that workers associate a specific time with a specific place but also that they estimate the relative duration of the reward period. In fact, ants repeatedly visit the expected reward site during at least the 1 h corresponding to the reward period. They start to visit the appropriate site approximately 30 min before food becomes available and continue to visit this site for a short while after the period of food availability has elapsed. The anticipatory visits before the onset of reward should not be interpreted as indicating imprecision in the time sense of the ants. They are also observed in bees (e.g. von Frisch, 1965; Koltermann, 1974) and may function to prevent competitors from pre-emptively depleting the food source; we observed such competition between different insect species in the field during the present study. Moreover, the complete or partial absence of reward is associated with a significant increase in the trip duration. These findings suggest that ants, under these particular conditions, do not use a 'win–stay, lose–shift' strategy because they remain at the unrewarded but correct site during the expected period of food availability rather than moving to a different site. Thus, taken together, these observations support the hypothesis that ants, like honeybees, do not rely on an immediate reward to control their behaviour,

as is generally assumed for many insect species, but that like several vertebrate species they can encode and store spatial or temporal information (Staddon, 1983; Gallistel, 1990; Beugnon et al., 1996; Menzel et al., 1998).

This research suggests that the ecological and evolutionary significance of spatio-temporal learning should be investigated more systematically by comparing the learning performances of different species. It is known for ants that individuals or colonies develop expectations about where or when to find food (for a review, see Nonacs and Soriano, 1998). Our results explain how the sugar collectors of *E. ruidum* can exploit the secretions of extrafloral nectaries under natural conditions (Weber, 1946; Lachaud et al., 1984; Jaffe et al., 1989; Passera et al., 1994), which are produced only at restricted times of the day and generally die within 1 week (Passera et al., 1994). While two neotropical ant species can learn when nectar is available (present data; Harrison and Breed, 1987), 15 temperate species showed no such ability, even after extensive training (Reichle, 1943; Dobrzanski, 1956; Fourcassié and Traniello, 1994). A possible ecological correlation is that none of these temperate species relies on nectar sources with regularly timed production (Carroll and Janzen, 1973; Cruden et al., 1983); similar correlations occur among insectivorous and granivorous bird species (Falk et al., 1992). Our experiments therefore illustrate the fact that biological constraints and evolutionary selective forces acting on learning processes can be quite similar across separate phylogenetic groups. This could explain why some insect species have been found to be capable of complex information processing and learning thought to be found only in vertebrate species (Beugnon and Lachaud, 1992; Chameron et al., 1998; Menzel, 1985, 1990). For example, despite 30 days of training, a cichlid fish *Cichlasoma nigrofasciatum* did not associate the location of a food site with a specific time of day (Reebs, 1993). Instead, the fishes learnt which sites provided food at any time of the day and visited them successively after the feeding signal was given. Other experiments with a different fish species, *Notemigonus crysoleucas*, indicate that Cyprinidae can learn to associate only two places, but not three, with different times (Reebs, 1996). The unchanged spatio-temporal pattern of visits performed by ants in the absence of reward in the present study demonstrates a spatio-temporal memory for each site separately. Similar results obtained in garden warblers suggest the use of time–place representations (Krebs and Biebach, 1989), whereas rats may use an ordinal timer in a daily time–place learning task, i.e. sequential access to the spatio-temporal information (Carr and Wilkie, 1997). How animals learn to regulate their foraging activity under time-based reward schedules is a good means to investigate how memory is organised in those species (Wilkie et al., 1997). The results, however, may depend on the reward schedule and test procedure used in the experiments. The joint spatio-temporal control of behaviour described here for the ant *E. ruidum* should promote more comparative vertebrate–invertebrate studies in the field of spatial and temporal cognition, especially considering that animals possess a battery of different problem-

solving mechanisms that operate within the temporal and spatial domains (Carr and Wilkie, 1997).

References

Beling, I. (1929). Über das Zeitgedächtnis der Bienen. *Z. Vergl. Physiol.* **9**, 259–338.

Beugnon, G. and Lachaud, J. P. (1992). La représentation chez l'insecte: au commencement était l'image. *Psychol. Française* **37**, 21–28.

Beugnon, G., Pastergue, I., Schatz, B. and Lachaud, J. P. (1996). Cognitive and behaviouristic approaches of space and time information processing in insects. *Behav. Process.* **35**, 55–62.

Biebach, H., Gordijn, M. and Krebs, J. R. (1989). Time-and-place learning by garden warblers, *Sylvia borin. Anim. Behav.* **37**, 353–360.

Biebach, H., Krebs, J. R. and Falk, H. (1994). Time–place learning, food availability and the exploitation of patches in garden warblers, *Sylvia borin. Anim. Behav.* **48**, 273–284.

Carr, J. A. R. and Wilkie, D. M. (1997). Rats use an ordinal timer in a daily time–place learning task. *J. Exp. Psychol. Anim. Behav. Proc.* **23**, 232–247.

Carroll, C. R. and Janzen, D. H. (1973). Ecology of foraging by ants. *Annu. Rev. Ecol. Syst.* **4**, 231–258.

Chameron, S., Schatz, B., Pastergue-Ruiz, I., Beugnon, G. and Collett, T. S. (1998). The learning of a sequence of visual patterns by the ant *Cataglyphis cursor. Proc. R. Soc. Lond. B* **265**, 2309–2313.

Cruden, R. W., Hermann, S. M. and Peterson, S. (1983). Patterns of nectar production and ant-pollinator coevolution. In *The Biology of Nectaries* (ed. B. Bentley and T. Elias), pp. 80–125. New York: Columbia University Press.

Dobrzanski, J. (1956). Badania nad zmystem czasu u mrowek. *Folia Biol. Krakow* **4**, 385–397.

Falk, H., Biebach, H. and Krebs, J. R. (1992). Learning a time–place pattern of food availability: a comparison between an insectivorous and a granivorous weaver species (*Ploceus bicolor* and *Euplectes hordeaceus*). *Behav. Ecol. Sociobiol.* **31**, 9–15.

Fourcassié, V., Schatz, B. and Beugnon, G. (1999). Temporal information in social insects. In *Information Processing in Social Insects* (ed. C. Detrain and J. L. Deneubourg). Basel: Birkhauser (in press).

Fourcassié, V. and Traniello, J. F. A. (1994). Food searching behaviour in the ant *Formica schaufussi* (Hymenoptera, Formicidae): response of naive foragers to protein and carbohydrate food. *Anim. Behav.* **48**, 69–79.

Franklin, C. (1994). Ants are smart enough to keep appointments. *New Scientist* **140**, 15.

Frisch, B. and Koeniger, N. (1994). Social synchronization of the activity rhythms of honeybees within a colony. *Behav. Ecol. Sociobiol.* **35**, 91–98.

Gallistel, C. R. (1990). *The Organization of Learning*. Cambridge. MA: MIT Press.

Harrison, J. M. and Breed, M. D. (1987). Temporal learning in the giant tropical ant, *Paraponera clavata. Physiol. Ent.* **12**, 317–320.

Herrera, C. M., Herrera, J. and Espadaler, X. (1984). Nectar thievery by ants from southern Spanish insect-pollinated flowers. *Insectes Soc.* **31**, 142–154.

Jaffe, K., Pavis, C., Vansuyt, G. and Kermarrec, A. (1989). Ants visit extrafloral nectaries of the orchids *Spathoglotis plicata* Blume. *Biotropica* **21**, 278–279.

Koltermann, R. (1974). Periodicity in the activity and learning performance of the honey bee. In *Experimental Analysis of Insect Behaviour* (ed. L. B. Browne), pp. 218–227. Berlin: Springer Verlag.

Krebs, J. R. and Biebach, H. (1989). Time–place learning by garden warblers (*Sylvia borin*): route or map? *Ethology* **83**, 248–256.

Lachaud, J. P. (1985). Recruitment by selective activation: an archaic type of mass recruitment in a ponerine ant (*Ectatomma ruidum*). *Sociobiology* **11**, 133–142.

Lachaud, J. P. (1990). Foraging activity and diet in some neotropical ponerine ants. I. *Ectatomma ruidum* Roger (Hymenoptera, Formicidae). *Folia Ent. Mex.* **78**, 241–256.

Lachaud, J. P., Fresneau, D. and Garcia-Perez, J. (1984). Etude des stratégies d'appro-visionnement chez trois espèces de fourmis ponérines. *Folia Ent. Mex.* **61**, 159–177.

Medugorac, I. and Lindauer, M. (1967). Das Zeitgedächtnis der Bienen unter dem Einfluß von Narkose und von sozialen Zeitgebern. *Zeit. Vergl. Physiol.* **55**, 450–474.

Menzel, R. (1985). Learning by honey bees in an ecological and behavioural context. In *Experimental Behavioural Ecology* (ed. B. Hölldobler and M. Lindauer), pp. 55–74. Stuttgart: Gustav Fischer Verlag.

Menzel, R. (1990). Learning, memory and 'cognition' in honey bees. In *Neurobiology of Comparative Cognition* (ed. R. P. Kesner and D. S. Olten), pp. 237–292. Hillsdale, NJ: Erlbaum Inc.

Menzel, R., Geiger, K., Joerges, J. and Chittka, L. (1998). Bees travel novel homeward routes by integrating separately acquired vector memories. *Anim. Behav.* **55**, 139–152.

Moore, D., Siegfried, D., Wilson, R. and Rankin, M. A. (1989). The influence of time of day on the foraging behavior of the honeybee, *Apis mellifera. J. Biol. Rhythms* **4**, 305–325.

Nonacs, P. and Soriano, J. L. (1998). Patch sampling behaviour and future foraging expectations in Argentine ants, *Linepithema humile. Anim. Behav.* **55**, 519–527.

Passera, L., Lachaud, J. P. and Gomel, L. (1994). Individual food source fidelity in the neotropical ponerine ant *Ectatomma ruidum* Roger (Hymenoptera Formicidae). *Ethol. Ecol. Evol.* **6**, 13–21.

Pratt, S. (1989). Recruitment and other communication behavior in the ponerine ant *Ectatomma ruidum. Ethology* **81**, 313–331.

Reebs, S. G. (1993). A test of time–place learning in a cichlid fish. *Behav. Process.* **30**, 273–281.

Reebs, S. G. (1996). Time–place learning in golden shiners (Pisces: Cyprinidae). *Behav. Process.* **36**, 253–262.

Reichle, F. (1943). Untersuchungen über Frequenzrhythmen bei Ameisen. *Z. Vergl. Physiol.* **30**, 227–251.

Rijnsdorp, A., Daan, S. and Dijkstra, C. (1981). Hunting in the kestrel, *Falco tinnunculus* and the adaptive significance of daily habits. *Oecologia* **50**, 391–406.

Saksida, L. M. and Wilkie, D. M. (1994). Time-of-day discrimination by pigeons, *Columba livia. Anim. Learning Behav.* **22**, 43–154.

Schatz, B., Beugnon, G. and Lachaud, J. P. (1994). Time–place learning by an invertebrate, the ant *Ectatomma ruidum* Roger. *Anim. Behav.* **48**, 236–238.

Schatz, B., Beugnon, G. and Lachaud, J. P. (1995a). Time representation in a neotropical ant *Ectatomma ruidum* Roger. In *Proceedings of European Conference of Cognitive Sciences, ECCS '95*, St Malo, France, pp. 137–144.

Schatz, B., Beugnon, G. and Lachaud, J. P. (1996). Polyethism within hunters of the ponerine ant, *Ectatomma ruidum* Roger (Hymenoptera, Ponerinae). *Insectes Soc.* **43**, 111–118.

Schatz, B., Lachaud, J. P. and Beugnon, G. (1993). Apprentissage temporel chez la ponérine néotropicale: *Ectatomma ruidum* Roger. *Actes Coll. Insectes Soc.* **8**, 9–15.

Schatz, B., Lachaud, J. P. and Beugnon, G. (1995b). Spatial fidelity and individual foraging specializations in the neotropical ponerine ant, *Ectatomma ruidum* Roger (Hymenoptera; Formicidae). *Sociobiology* **26**, 269–282.

Schatz, B., Lachaud, J. P. and Beugnon, G. (1997). Graded recruitment and hunting strategies linked to prey weight and size in the ponerine ant, *Ectatomma ruidum. Behav. Ecol. Sociobiol.* **40**, 337–349.

Staddon, J. E. R. (1983). *Adaptive Behavior and Learning.* Cambridge: Cambridge University Press.

Terborgh, J. (1983). *Five New World Primates. A Study in Comparative Ecology.* Princeton: University Press.

von Frisch, K. (1965). *Tanzsprache und Orientierung der Bienen.* Berlin: Springer Verlag.

Wahl, O. (1932). Neue Untersuchungen über das Zeitgedächtnis der Bienen. *Z. Vergl. Physiol.* **16**, 529–589.

Weber, N. A. (1946). Two common ponerine ants of possible economic significance, *Ectatomma tuberculatum* and *E. ruidum. Proc. Ent. Soc.* **48**, 1–16.

Wenger, D., Biebach, H. and Krebs, J. R. (1991). Free running circadian rhythm of a learned feeding pattern in starlings. *Naturwissenschaften* **78**, 87–89.

Wilkie, D. M., Carr, J. A. R., Galloway, J., Parker, K. J. and Yamamoto, A. (1997). Conditional time–place learning. *Behav. Process.* **40**, 165–170.

Wilkie, D. M., Carr, J. A. R., Siegenthaler, A., Lenger, B., Liu, M. and Kwok, M. (1996). Field observations of time–place behaviour in scavenging birds. *Behav. Process.* **38**, 77–88.

Wilkie, D. M., Sakisda, L. M., Samson, P. and Lee, A. (1994). Properties of time–place learning by pigeons, *Columbia livia. Behav. Process.* **31**, 39–56.

Wilkie, D. M. and Willson, R. J. (1992). Time–place learning by pigeons, *Columba livia. J. Exp. Anal. Behav.* **57**, 145–158.

Wilkie, D. M. and Willson, R. J. (1995). More evidence of robust spatial associative memory in the pigeon, *Columba livia. Anim. Learn. Behav.* **23**, 69–75.