

HOW DO *BLATTELLA GERMANICA* (L.) AGGREGATE?

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Abstract - German cockroaches, *Blattella germanica* (L.), live in aggregates and are able to return to their resting sites. Stability of aggregates is ensured both by individual navigation abilities and by conspecific recognition. Navigation capacities (estimated by orientation angle of escape direction and by length of escape path) are due to two types of memorization processes: short-term memory with path integration cues learned during each trip and long-term memory of landmark cues in their home range. The different types of directional information used for homing are weighted and used in complement in relation to their reliability. To pinpoint their shelter, they use pheromonal cues to recognize conspecifics. Experiments revealed that cockroaches were able to discriminate and recognize the odour of members of their own strain and avoid odours of unknown strains and that cuticular hydrocarbons operate as a recognition signal allowing proximity tolerance between individuals, i.e. as an aggregation pheromone.

Key words - Navigation, social recognition, aggregation, cockroach

INTRODUCTION

The questions raised in this type of study aim at obtaining a better knowledge of how cockroaches live in our urban habitat so that this might, in the end, help us to understand why they are so successful in spite of all our efforts to get rid of them. *Blattella germanica* cockroaches, for example, live in aggregates centred around a shelter (Rivault, 1989). Within an area, they move about for many reasons: in search of food, a potential mate, a shelter, to explore new territories, to interact with competitors or to escape from a predator. The precision with which all these tasks are performed is not due to random events. All species have developed behaviour adapted to their environment. The probability of arriving at a particular site or of finding a relevant target is improved if an insect can use information that can guide it to its goal (Gallistel, 1990; Lehrer, 1997). Insects like cockroaches keep returning to already known, particular sites, whether, for example, these sites are profitable food sources or resting sites.

We tried to understand the different types of mechanisms used during these types of oriented behaviour. We wanted to know the part played by different types of information perceived by the insect, at its level, and how this information shapes individual behavioural decisions. Different types of factors contribute to the spatial stability of cockroach aggregates. While navigating, cockroaches perceive and use visual, vibratory, tactile and chemical cues which can have either an environmental or a conspecific origin and act as recognition signals. Stability of *Blattella germanica* aggregates is ensured both by individual navigation abilities and by conspecific recognition that maintain each individual within its aggregate.

The aim of this study was to understand the part played by different categories of spatial information in controlling short distance homing in the cockroach *Blattella germanica* (L.) during its return journey to its shelter and to analyse the weighting of different information sources and to demonstrate the role of chemical cues in group member recognition.

MATERIALS AND METHODS

Individual navigation

The success of a return path depends on the correct estimation of two components: orientation angle and estimation of distance to the goal. To demonstrate the use of path integration cues and of learned landmark cues in estimating orientation angle, we used the following standard experimental conditions.

Cockroach larvae were allowed to move freely between their shelter, at the edge of the test box, and a food dish, in the centre of the box, for three days after hatching. On the fourth day, the test measured the escape direction of larvae from the food dish following a disturbance. The predicted escape direction was the shelter direction, shelter which is a safe refuge. Tests were performed at the beginning of the dark phase of the light-dark cycle, during the cockroaches' main activity peak. Escape paths were recorded with a camera equipped with an infra-red light. Recordings were analysed with an image processing software developed in the laboratory (Dabouineau and Rivault, 1994).

To demonstrate that they were able to estimate distance correctly with path integration cues collected during the outward trip, cockroaches were tested in a large arena, in complete darkness. The test situation was similar to that previously described. While they were feeding in a corner of the arena, their cardboard shelter was removed. Feeding cockroaches were then disturbed to make them flee towards their shelter. To avoid any interference with scent marks that might have been deposited around the shelter, the paper covering the floor of the arena was changed just before a test. The return paths were videotaped and analysed using an image processing software (Durier and Rivault, 1998).

Conspecific recognition

When an individual reaches the vicinity of its shelter using navigation processes, pheromonal cues are used to pinpoint the goal. To demonstrate that cockroaches use chemical cues to recognize their shelter, we used the following standard experimental conditions. Our test situation was similar to that described by Ishii and Kuwahara (1967). A group of 20 first instar larvae placed in a petri dish was presented with a choice between two filter papers. These papers, placed up-right on the dish were either clean or conditioned with different odours. As larvae aggregate when resting, we recorded their positions after an active phase, during the following rest period. Tests lasted 24 hours. Larvae could be in three different positions: on either of the two papers or elsewhere on the dish. Papers were conditioned by body contact of larvae, always under strictly identical conditions (number of larvae and duration of conditioning) (Rivault and Cloarec, 1998).

RESULTS AND DISCUSSION

Individual navigation

Insects use different spatial information sources to navigate within their familiar home range, relying on non-learned stimuli like taxis (Kühn, 1919), on genetically determined preferences (Pardi, 1960; Papi and Tongiorgi, 1963) (like attractiveness to dark shaded areas, or scototaxis, in cockroaches), or else on memorized stimuli which can refer either to short-term memory in the case of self-generated signals through path integration processes or to long-term memory in the case of learned environmental landmarks (Beugnon, 1986). The short-term memory process called path integration remains, by definition, available only within a given sequence of locomotor activity. A path integration system enables an animal to direct its locomotion without using landmarks, by making use of information provided by its own previous movement sequences or its previous positions, which are collected through exoskeleton sense organs (Seyfarth *et al.*, 1982; Mittelstaedt, 1983; Wehner and Wehner, 1986). At any time, kinesthetic cues provide the animal with a continuous egocentric representation of its position relative to its starting point, including direction and distance (Seyfarth and Barth, 1972; Seguinot *et al.*, 1993). After having travelled along a sinuous path, this representation allows an insect to evaluate the orientation angle and the distance to travel to return to its shelter in a straight line. Long-term memory processes occur when stable configurations in the outside world allow a subject to organize its environment on a long-term basis and to learn conspicuous landmarks around its shelter for example. An animal relates changes in the perception of different cues to its own movements and can thus organize its visual environment. The correlation between preceding movements and expected changes in its visual field reinforce the recognition of a given place (Etienne *et al.*, 1990).

When, in a control experiment, a dim light allowed larvae to see landmarks during the night phase, they could learn landmark cues. When a shake disturbed them while they were feeding, they escaped towards their shelter. Thus they could rely on both types of cues: path integration cues taken during the outward trip and visual cues learned during previous trips made during the days preceding the test. The escape direction was significantly oriented towards the shelter (Fig. 1, exp. 1) (Dabouineau and Rivault, 1994). The experimental conditions described here were typical of their usual life conditions in a kitchen for example, where they usually benefit from dim lighting during their nocturnal phase of activity.

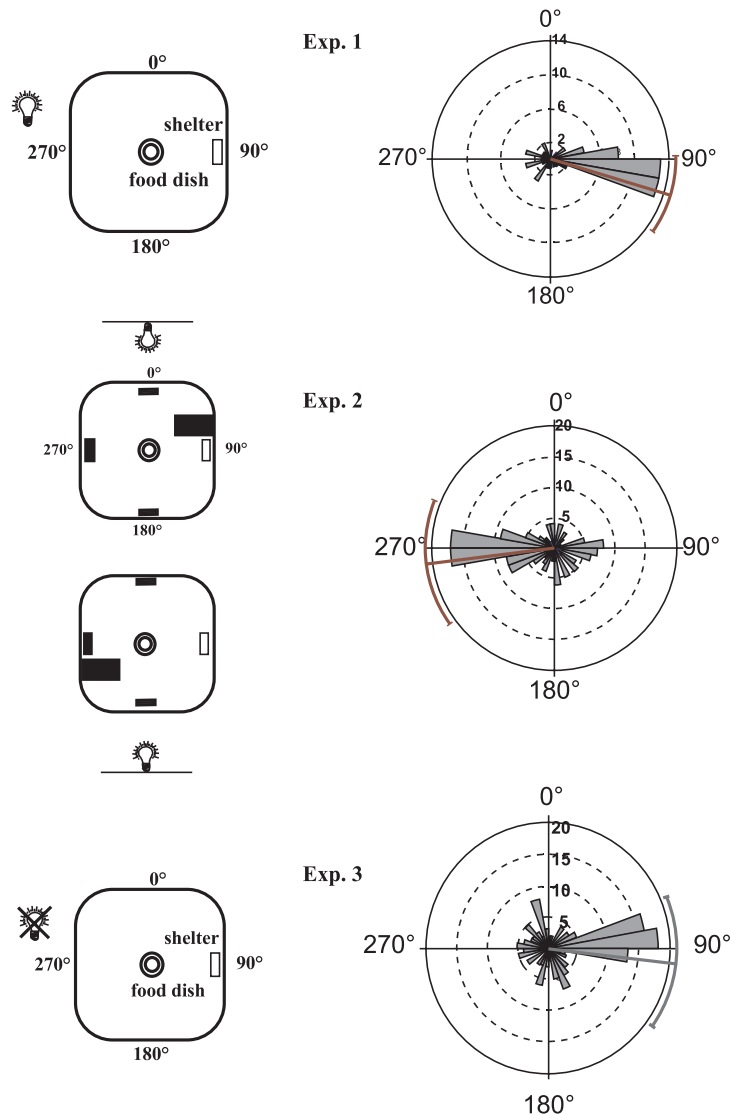


Figure 1. Diagramme of experimental procedure and distribution of angular direction chosen by cockroach larvae after a disturbance on the food dish. Expected escape angle is always 90°. Length of histogramme bars referred to the number of larvae in 10° classes. Statistical analyses of data were obtained by using Oriana software (Kowach computing System). Angle of mean vector and angular deviation are indicated by a black arrow. N: number of larvae, f: mean escape orientation angle, r: mean vector length, d: angular deviation, p: significance level of Raleigh test. Exp. 1: larvae have a dim non-directional light during the night phase to learn the landmark cues. After the disturbance, they can use path integration cues plus learned landmark cues to return to their shelter. N= 68, f=107°, r=0.49, d=18°, p<0.001. Exp. 2: larvae have a directional dim light plus a black landmark cue to indicate their shelter position. After the disturbance, they can use either their path integration cues or the shifted landmark cues to return to their shelter. N= 157, f= 263°, r=0.22, d=28°, p<0.001. Exp. 3: larvae are in complete darkness so that they can use only path integration cues to return to their shelter. N= 162, f= 99°, r= 0.21, d=29°, p<0.001.

To demonstrate the part played by each of these mechanisms, further experiments were made. The importance of landmark learning in shelter orientation was revealed by rotating learned landmark cues during the test. Before a test, a black landmark was placed near the shelter and a light directed towards one side of the experimental room, these cues enabled larvae to learn their shelter direction. The position of both the directional light and the black landmark were shifted 180° just before a test. When disturbed, larvae did not orientate towards their shelter but in the opposite direction. They followed the conspicuous learned landmark cues and missed their shelter (Fig. 1, exp. 2) (Rivault and Dabouineau, 1996).

To demonstrate the importance of path integration cues in this escape behaviour, tests were carried out in complete darkness so that larvae could no longer rely on landmark cues, but only on their path integration cues. The results of this experiment revealed significant orientation towards the shelter (Fig. 1, exp. 3) (Durier and Rivault, 1998).

Most return paths were characterized by a nearly linear trajectory oriented towards the goal at quick speed, an arrest of this oriented trajectory and a final long-lasting sinuous search at much slower speed (Fig. 2). The length of the escape path up to the arrest position represented the distance cockroaches estimated necessary to reach their shelter. When they failed to find their shelter at the arrest position, they adopted another search strategy. The final part of their search path was centred around the arrest position as if the cockroaches were looping around it, increasing the size of their loops with time. In these experiments no external cues were available for navigation and cockroaches relied exclusively on information from their own movements, provided by sense organs located in their legs (Durier and Rivault, 1998).

These results indicate that individuals are able to return to the shelter area occupied by their aggregate after a foraging trip. Their navigation capacities are due to their knowledge of their familiar environment. This knowledge is mainly due to two types of memorization processes: short-term memory with path integration cues learned during each foraging trip and renewed during each trip. Cockroaches also use long-term memory with learning of landmark cues in their home range, particularly around their shelter. Both mechanisms are used in complement and help each individual to orientate and to evaluate the distance that separates it from its resting area after a foraging trip. Nevertheless, when some environmental cues are modified during their trip, they analyse the new situation and change the relative weighting of different types of directional information used for homing in relation to the reliability of the different types of cues, switching from one mechanism to the other in relation to environmental conditions.

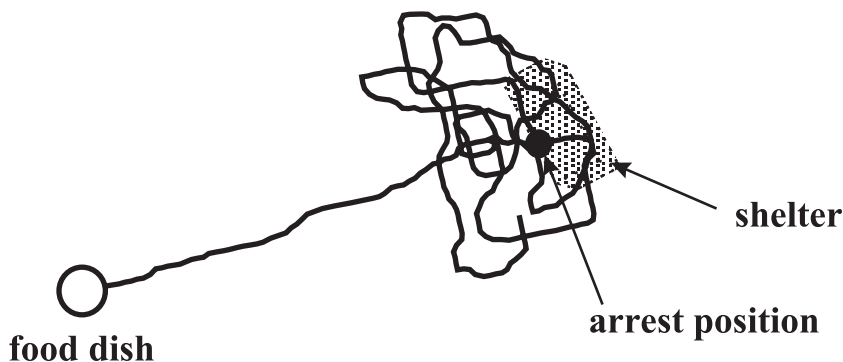


Figure 2. Details of a typical escape path from the food dish to the shelter area after a disturbance, characterized by a nearly linear trajectory oriented towards the goal at quick speed, an arrest position indicating the estimated distance of the goal and a final long-lasting sinuous search at much slower speed.

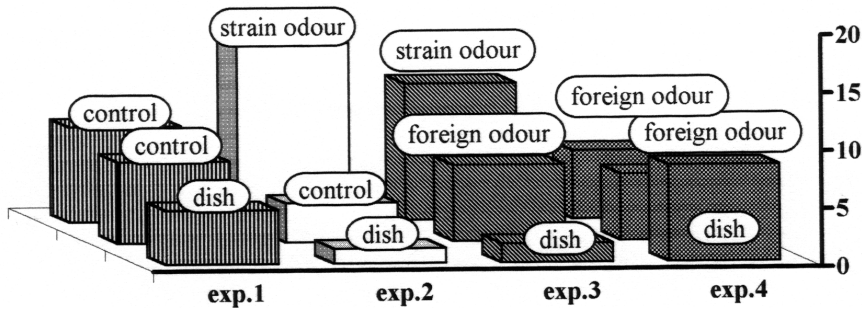


Figure 3. Mean distribution of groups of 20 larvae in choice tests between two papers differently conditioned. Larvae not attracted on the papers remained on the dish. Exp. 1: choice between two control papers. Exp. 2: choice between a control paper and a paper conditioned by their own strain odour. Exp. 3: choice between a paper conditioned by their own strain odour and a paper conditioned by a foreign strain odour. Exp. 4: choice between two papers conditioned by two different foreign strain odours.

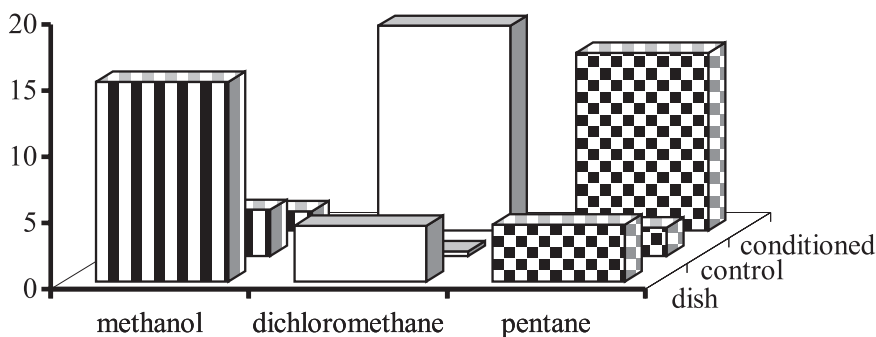


Figure 4. Mean distribution of groups of 20 larvae in choice tests between a control paper and a paper conditioned with extracts obtained with one of three different solvents: methanol, dichloromethane and pentane.

Conspecific recognition

The second type of behavioural trait contributing to the stability of aggregates is pheromonal communication and recognition between group members. Although there is a long history of attempts to identify and to locate the source of secretion of the aggregation pheromone in this species, important discrepancies between previous results led us to undertake a completely new behavioural analysis (Ishii and Kuwahara, 1967; MacFarlane and Alli, 1986; Sakuma and Fukami, 1990, 1991, 1993; Rivault *et al.*, 1998).

Larvae were first presented two clean papers which acted as controls. This experimental situation tested the attraction of filter papers in the absence of any odour. The larvae were attracted to clean papers and preferred to rest on papers rather than on the plastic dish. They were able to aggregate on clean papers in the absence of any attractive odour. They all aggregated together, either on the left or on the right paper but showed no preference for either paper, as indicated by the mean number of larvae in each position for all tests (Fig. 3). Larvae were then given a choice between a clean paper and a paper conditioned by their own strain odour. Larvae preferred a conditioned paper to a clean paper. The number of larvae that stayed on the dish was significantly lower than in the previous experiment. Conditioned papers attracted, or more exactly, arrested, more larvae than a clean paper.

Larvae were given a choice between a paper conditioned with their own odour and a paper conditioned with the odour of conspecific larvae from another strain. We consider that a strain is a group of cockroaches captured at a given location, well separated from other cockroaches. All captured strains were reared under identical climatic conditions in the laboratory and fed the same diet. Larvae preferred paper conditioned by their own strain odour to paper conditioned with the odour of another strain. Then larvae were given a choice between two papers conditioned with the odour of two unfamiliar strains. They did not prefer either odour. Approximately one third of the larvae was recorded on one paper, another third on the second paper and last third remained on the dish. The number of larvae that stayed on the dish was particularly important. The distribution of the larvae appeared to be random and was not the result of any attraction to a particular position.

Larvae were given a choice between a control paper and a conditioned paper (Fig. 4) (Rivault *et al.*, 1998). Papers were no longer conditioned by direct body contact of larvae but by cuticular hydrocarbon extracts in different solvents. Three solvents were used: dichloromethane, methanol, and pentane. The results of choice tests showed that larvae were significantly attracted onto papers conditioned with either dichloromethane or pentane extracts, but were not attracted onto papers conditioned with methanol extracts. Two solvents were efficient in extracting active substances able to induce aggregation behaviour. The analysis of these extracts by gas chromatography revealed that all the components extracted were cuticular hydrocarbons (C27 to C32).

Social factors contribute to aggregate stability. Behavioural tests confirmed the role of attractive substances deposited by body contact in the choice of the position of aggregates in the environment. Olfactory cues acting only at short distance are perceived and secreted by cockroaches of all age classes. Choice tests comparing the attractiveness of odours between strains from well-separated geographical locations revealed that cockroaches were able to discriminate and recognize the odour of members of their own strain and avoid odours of unknown strains. Odours vary between strains. We have also shown that cuticular hydrocarbons operate as a recognition signal allowing proximity tolerance between individuals, i.e. as an aggregation pheromone in *Blattella germanica*. As there is plenty of evidence that cuticular hydrocarbons play an important role in intra- and inter-colony recognition in social insects like termites and ants, further experiments will be necessary to understand the mechanism of strain recognition in *Blattella germanica* as well as its function.

CONCLUSION

The cues that guide cockroaches may be provided by path integration cues, learned landmark cues placed along the route to that goal and near the goal itself. Once they have arrived, the insects have to identify the target. The identification task can imply various sensory modalities like vision or smell and they need not be the same as those used during the journey. Individual navigation abilities and strain recognition both contribute to the stability of aggregates in the sense that each individual returns to its resting area after a foraging trip, and is able to recognize the odour of its group members.

These experimental results and the fundamental analysis of aggregation behaviour in *Blattella germanica* cockroaches demonstrate that they are able to navigate very precisely over their home range and consequently they are able to exploit very efficiently the resources that are offered in urban habitations. When something is modified in their home range, like the introduction of a trap, a food bait, or an insecticide treated area, they are able to identify it as a novelty. These modifications are then integrated into a new visual snapshot of their surroundings and will be taken into account during the next trips. According to the position of the new item inside the cockroach's home range, its presence can be analysed differently. For example, proximity with an already known landmark cue or its position along a known path leading from a shelter to a food source, will not have the same meaning if cockroaches find the new item near a food source or near their shelter. This could modify the issue of the expected control of the population.

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