J. Insect Physiol. Vol. 29, No. 7, pp. 575 to 579, 1983
Printed in Great Britain.

0022-1910/83$03.00 +0.00

SEARCH STRATEGY OF SEX PHEROMONE-STIMULATED MALE GERMAN COCKROACHES

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(*Received* 23 *November*, 1982)

Abstract—Stimulation of the male German cockroach, *Blattella germanica*, with contact sex pheromone releases courtship behaviour and then area-restricted local search. Servosphere-recorded search patterns, compared to pre-stimulus pathways, are characterised by increased looping and path crossing and a relatively high rate of turning. Search behaviour can be performed in the absence of visual cues, including information relative to the site of stimulation. Information controlling search motor patterns is at least in part genetic; certain pattern components are generally similar from trial to trial, whereas specific pattern components vary widely.

Key Word Index: Cockroach, *Blattella germanica*, orientation, search, mate finding, sex pheromone

INTRODUCTION

THE FEMALE sex pheromone of the German cockroach, *Blattella germanica*, has been shown to be a mixture of 3,11-dimethyl-2-nonacosanone (compound A) and 29-hydroxy-3,11-dimethyl-2-nonacosanone (compound B) (NISHIDA *et al.*, 1975, 1976; ROSENBLUM *et al.*, 1976; SCHWARTZ *et al.*, 1975; BURGSTAHLER *et al.*, 1975, 1977). A 3:1 blend of these compounds proved most effective in eliciting male courtship activity (BURGSTAHLER *et al.*, 1977). That the pheromone is relatively non-volatile was shown earlier by ROTH and WILLIS (1952) in their analysis of the stimuli involved in courtship of this species. This prompted NISHIDA *et al.* (1975) to develop a bioassay for synthetic pheromones based on contact chemoreception.

Search behaviour has been well studied in systems where an animal locates a resource and then engages in area-restricted search in order to find other samples of the same resource. Most studies have dealt with optimal foraging strategies of predators or parasitoids seeking hosts (reviews: HASSELL, 1978; WESELOH, 1981; VINSON, 1983) or herbivores seeking plants or plant products (reviews: PYKE *et al.*, 1977; MILLER and STRICKLER, 1983). We are not aware of any quantitative study that involves search for potential mates without utilizing external sensory information.

The present report deals with the behaviour of males in close proximity to a sex pheromone source and male search behaviour stimulated by antennal contact with a pheromone source. The results partially elucidate the mate-finding strategy of the male German cockroach which appears to operate in the absence of volatile cues.

MATERIALS AND METHODS

Insect material

Blattella germanica colonies were maintained as described by BELL *et al.* (1978a). Males were isolated from females for 7–9 days prior to experimentation.

Pheromone application techniques

A 50:50 mixture of synthetic compound A and B was employed at a concentration of 10 μg/μl (BELL *et al.*, 1978a,b). Previous studies have shown that courtship behaviour of the German cockroach includes antennal fencing of the female by the male. Antennation presumably allows the male to perceive sex pheromone on the cuticular surface of the female (ROTH and WILLIS, 1952; BELL and SCHAL, 1980). The method used to simulate contact of the female by the male employs an ablated antenna from male *Periplaneta americana* to eliminate possible non-chemical stimuli specific to German cockroaches. A freshly ablated antenna, fixed to a glass rod, was dipped into a pheromone–carbon tetrachloride solution for 1–2 sec and allowed to dry for 30 sec prior to use. The antenna was then touched to both antennae of a male cockroach for 1–2 sec.

Analysis of search behaviour

A male *B. germanica* was allowed to walk on a servosphere apparatus (BELL *et al.*, 1983b; see also WEBER *et al.*, 1981) for 15 min to accommodate to the locomotion compensation device. The experiment was performed in total darkness; an infra-red viewer was used to observe the cockroach and to allow application of sex pheromone to its antennae. After the 15-min accommodation period and a 2-min recorded pre-stimulus period, the antennae of the free-running cockroach were stroked with a pheromone-impregnated antenna.

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Locomotion of cockroaches on the servosphere was translated into incremental X and Y co-ordinates by two optical shaft encoders positioned 90° apart against the sphere. A minicomputer stored pairs of X/Y co-ordinates at 0.1 sec intervals in memory and simultaneously displayed the resulting orientation pathway in two dimensions on a graphics terminal (SURBER, 1982). The path of the cockroach was recorded for 2 min prior to pheromone application and then for 2 min after pheromone was applied. The paths were analysed with computer programmes and reproduced on an X–Y plotter.

The following indexes were calculated: (1) linearity index, a measure of path straightness, calculated by dividing the distance between the first and last points (i.e. beeline) by the path length, (2) number and duration of stop periods and total stop period during a run, (3) mean vector, r, for each path, a measure that is unaffected by the length of the beeline, (4) mean locomotory speed (cm sec^{-1}), (5) absolute turning velocity, expressed as degrees turned (summing left and right turning components) per 1.0 sec interval, (6) relative turning rate, a measure of turn direction constancy, calculated by subtracting left and right turning components (e.g. relative turning rate equals zero if an animal moved straight or if it turned equally to the left and the right; a high relative turning rate results if an animal moved constantly either right or left, as in a spiral), (7) search intensiveness was quantified by calculating (a) the number of times an animal crossed its own path per m of pathway, (b) maximal distance, D-max, spanning the most distant points in a pathway, (c) number of loops and diameter of loops in a pathway. The area-of-search, diameters of loops, and number of times an animal crossed its own path, were calculated from hard-copy plots.

RESULTS

Behaviour of males in close proximity of a sex pheromone source

A group of 25 males was pre-tested using the impregnated antenna method. The results showed that 80% responded with courtship behaviour. On the following day a filter paper or glass plate, to which the same pheromone mixture was applied, was lowered into the chamber (14 × 24 × 30 cm) containing the males. The pheromone source was positioned over the males such that it was separated from their antennae by a distance of less than 4 mm. Changes in male behaviour and locomotory activity were monitored by close observation and by the line-crossing method (BELL, 1981b). The experiment was then repeated using a fine mesh screen upon which 10 virgin females were placed. Males in both situations showed no detectable responses during a 30-min assay period with regard to antennal waving, vertical orientation of the antennae or locomotory activity. The same males were then tested by stroking their antennae with the pheromone mixture. As on the previous day, 80% of the males responded with courtship behaviour, increased antennal movements and increased locomotory activity.

In another series of experiments, a virgin female was introduced into a group of 10 males in a cage. Observations were made as the female moved close to and passed males to determine if males responded to females in the absence of actual contact. Except in two cases where the female contacted a male, males did not orient to the passing female or engage in courtship activity.

These findings suggest that males do not respond to and apparently do not perceive sex pheromone transported in air. The studies using live virgin females indicate that additional sex pheromone components more volatile than the nonacosanone compounds, if present, do not augment the response.

Search pattern of males stimulated by contact with sex pheromone

Figure 1 illustrates to the same scale a pre-stimulus and a post-stimulus search pathway of a male *Blattella germanica.* Prior to stimulation with sex pheromone, males move in a relatively straight course (linearity index, 0.46 ± 0.24) on the servosphere, as previously noted by BELL and KRAMER (1979) (Figs. 2A–E). Wing-raising and a turn of approximately 180° occur on the sphere when the antennae of the male are stroked with the sex pheromone-impregnated antennae; these behaviour patterns occur while the cockroach is stopped at the beginning of an experiment (shown in figs as an open square). The male then lowers its wings and initiates local search (Fig. 2F–I). Males run a few cm after being stimulated, stop, turn approximately 120°–180°, and move toward the "site of stimulation" (Fig. 3); this kind of turning occurs throughout search patterns of some males (e.g. Fig. 2F, H and I). That search is restricted in area is shown by a decrease in D-max, an increase in path-crossings, and an increase in looping (Table 1). Intensive search is followed by reversion back to the type of locomotion exhibited prior to stimulation by sex pheromone. Because no visual cues are present in the

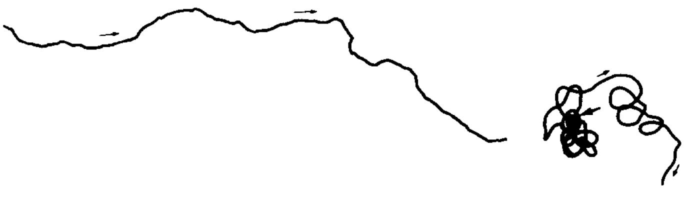

Fig. 1. Pathway of male cockroach recorded from a servosphere apparatus. In contrast to Fig. 2, paths before and after stimulation with sex pheromone are shown at the same scale. Arrow in post-stimulus path indicates point of stimulation; small arrows indicate direction of movement. The two paths are not connected because the recording device was disconnected during the 2 sec period while the male's antennae were being stimulated on the servosphere.

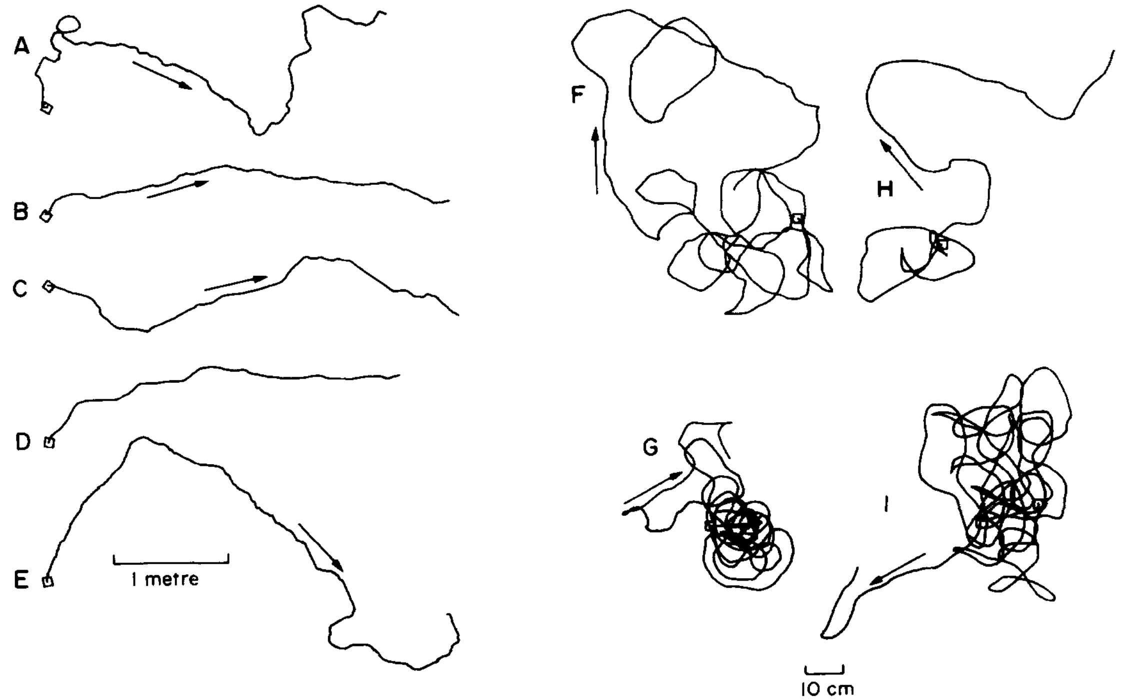

Fig. 2. Pathways of male cockroaches recorded from a servosphere apparatus for 2 min before (A–E) and after (F–I) stimulation with sex pheromone. Arrows indicate direction of movement from the starting point (shown as open square).

experimental setup, the cockroach cannot be orienting with respect to landmarks or other cues in the environment.

The search patterns are characterised by regular or irregular overlapping loops of 13.5 ± 7.4 cm dia (Table 1). Males stimulated by sex pheromone exhibited a decrease in linearity index and an increase in both absolute and relative turning rates. The locomotory rate while the animals actually moved remained the same; stimulated males, however, stopped for shorter periods and for less total stop-time than did the males prior to pheromone stimulation. Each search bout tended to be in one basic direction, either left or right (relative turning rate). The switch back to normal locomotory mode occurred gradually as depicted in Fig. 2.

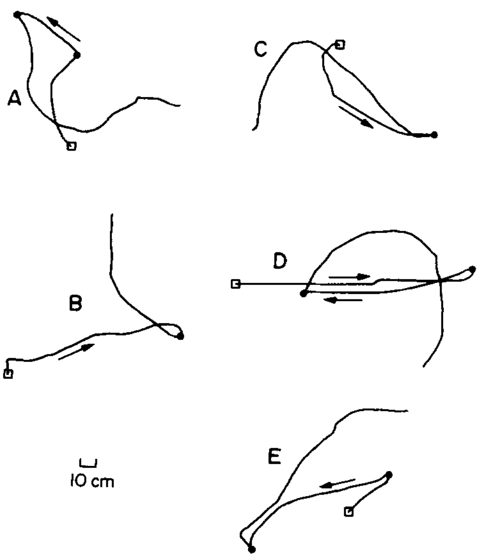

Fig. 3. Examples of initial 10 sec of orientation after stimulation. Arrows show direction of movement from startin point (open square). Solid dots indicate stopping points.

DISCUSSION

Volatile sex pheromones have been best studied among the potential sources of orientation information used for mate finding by cockroaches (reviews: Bell, 1981a; Breed, 1983; Schal *et al.*, 1983). The female sex pheromone of *P. americana*, for example, attracts males over relatively long distances (Tobin, 1981; Bell *et al.*, 1983a), and releases male search behaviour (Hawkins, 1978), upwind orientation (Rust and Bell, 1976; Tobin, 1981; Bell and Kramer, 1979), direct chemo-orientation (Rust *et al.*, 1976; Bell and Tobin, 1981), and chourtship behaviour (Roth and Willis, 1952). Schal (1982) recently showed that the vertical distribution of rain-forest cockroaches may be related to the pattern of dispersion of air-borne pheromones, a finding that may explain downward orientation of male *P. americana* when exposed to female sex pheromone (Silverman and Bell, 1979). *B. germanica* apparently has neither female nor male volatile sex pheromones, and cannot utilise the mechanisms described above to facilitate mate finding.

Based on experimental results presented here it seems that male *B. germanica* rely on chance encounters to locate females. The ranging strategy of males seems to be similar to that of walking starved

Table 1. Comparison of paths of males prior to and after stimulation with sex pheromone*

	Pre-stimulus path	Post-stimulus path	*P*†
Path straightness			
Linearity index ($\bar{x} \pm$ S.D.)	0.46 ± 0.24	0.12 ± 0.12	$P < 0.001$
Absolute turning rate ($\bar{x}$ degrees $\text{sec}^{-1} \pm$ S.D.)	23.9 ± 8.2	41.5 ± 14.3	$P < 0.01$
Mean vector ($\bar{x} \pm$ S.D.)	0.44 ± 0.22	0.12 ± 0.10	$P < 0.001$
Directional constancy			
Relative turning rate ($\bar{x}$ degrees $\text{sec}^{-1} \pm$ S.D.)	5.3 ± 5.9	11.6 ± 11.5	$0.05 < P < 0.1$
Search intensiveness			
Number of path crossings M^{-1}($\bar{x} \pm$ S.D.)	0.03 ± 0.07	0.73 ± 0.68	$P < 0.001$
D-max ($\bar{x} \pm$ S.D.)	355.5 ± 157.7	108.7 ± 56.8	$P < 0.001$
Number of loops ($\bar{x} \pm$ S.D.)	0.7 ± 1.3	5.6 ± 3.8	$P < 0.001$
Diameter of loops ($\bar{x}$ cm $\pm$ S.D.)	30.9 ± 21.9	13.5 ± 7.4	$P < 0.01$
Locomotory mode			
Locomotory rate ($\bar{x}$ cm $\text{sec}^{-1} \pm$ S.D.)	6.1 ± 1.7	5.9 ± 2.1	ns
Number of stops ($\bar{x} \pm$ S.D.)	14.4 ± 4.8	15.8 ± 6.2	ns
Duration of stops ($\bar{x}$ sec $\pm$ S.D.)	2.4 ± 1.4	0.6 ± 0.2	$P < 0.001$
Total stop period ($\bar{x}$ sec)	34.6	9.5	$P < 0.01$

* Based on 10 pathways of 5 male cockroaches. †Probability values based on Student's *t*-test, except for total stop period which was calculated from the Mann Whitney U-test.

predators (e.g. DIXON, 1959); ranging is characterised by high linearity and few turns (as in Figs 1, 2A–E, Table 1), whereas search after contact with prey is confined to a small area and consists of frequent turns and loops (as in Figs 1, 2F–I, Table 1). The probability of encounters with females is likely increased by the aggregative tendency of this species (e.g. ISHII and KUWAHARA, 1968). Field data (e.g. ROSS and WRIGHT, 1977; KEIL, 1981) indicate that *B. germanica* in domiciliary habitats are confined to specific, favourable harborages (sheltered, moist) where large groups may aggregate. Adults and nymphs of both sexes emit an aggregation pheromone which may act as an attractant or as an arrestant (review: BELL, 1981a). Thus it is probable that newly emerged virgin females occur in close proximity to males, and sexual communication over long distances is not required for mate-finding.

When a female has been detected by contact chemoreception, the male executes a single turn of approximately 180° (BELL and SCHAL, 1980; FRANKLIN *et al.*, 1981). It then begins to move in the area where it displayed, orientation which can be described as pheromone-released local or area-restricted search, whereby the male increases its chances of locating a female in the immediate vicinity. The orientation pattern cannot be directed by external cues, because the pheromone is not volatile and other possible cues were eliminated in the experimental design. It is highly unlikely that odour trails deposited on the sphere might be used for orientation cues because the sphere rotates even while an animal is executing a loop; thus a loop is never executed over the exact position of the sphere where a previous loop was made.

Local search of *B. germanica* males after contact with nonacosanone is similar to the "dancing" of blowflies and house-flies following the ingestion of a drop of sugar solution (DETHIER, 1957; NELSON, 1977; MURDIE and HASSELL, 1973; MOURIER, 1964). Prior to feeding there is an approximately equal probability of various turn angles and step lengths, whereas after the fly ingests sugar solution the local search pattern is characterised by larger turn angles. There is no relationship between the site of stimulation and the resulting orientation manoeuvre, since the pattern is elicited even after the fly is moved to a different place after it feeds (DETHIER, 1957).

In the case of parasitoids and predators (in the broad sense) area-restricted search occurs after prey are located, and is related to the density (or concentration) of the prey. A smaller area is searched when prey density is high (i.e. high encounter rate). In our design with *B. germanica*, temporary loss of the stimulus resulted in search of a confined area. The size of the area increased progressively with elapsed time after contacting a female; eventually the ranging pattern was resumed. The intensity of subthreshold stimuli determined the duration of local search (SCHAL, unpublished), as in flies where duration of search is related to concentration of sucrose imbided (NELSON, 1977). In our experiments with *B. germanica* no effort was made to simulate different female densities by applying various sequences of multiple stimulus patterns.

Both the "hungry fly" and the "enticed cockroach" exhibit orientation that is controlled by internally-derived, probably genetic, orientation information. The pattern is triggered by contact chemoreception, but controlled by the internal information. In both cases the behaviour functions to optimise the chances of the animal in locating other samples of the same type of resource in the immediate environment.

Acknowledgements—Supported by the National Science Foundation Psychobiology Program (BNS 82-03986), National Institutes of Health (NS 13798), and a University of Kansas Biomed allocation. The authors are indebted to Ernst Kramer for advice in constructing the servosphere apparatus.

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