Aggregation Pheromone of the German Cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae): Choice-Chamber Assay for Arrestant Component(s)¹

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Attractant amine hydrochlorides of the German cockroach aggregation pheromone were subjected to a vertical paper assay with contiguously standing sample and control papers. In spite of the potent attractiveness apparent in an olfactometer assay, the pheromone did not arrest nymphs more than the control. This suggested the presence of an arrestant component(s) other than the attractants. The assay was developed into a “choice-chamber” type, in which the floor of each cell was divided into sample and control areas. One test animal was confined in each cell, and after a standard time the preference was measured by the number of nymphs found in each area. Frass-contaminated paper arrested 89% of the nymphs at 20 min. Shadow and colour tone also influenced the preference, but tone of the frass-contaminated paper did not affect the result. The reduction of the activity by fine polyester gauze-covering suggested that the arrestant was a contact chemical. Assays on antenn- and/or palp ectomized male adults indicated that the arrestant was received with both antennae and maxillary palpi. Male adult bodies were dissected in parts and their methanol extracts were subjected to the assay. The arrestant was produced from the 10th abdominal tip (other than rectum).

Key words: aggregation pheromone, arrestant, attractant, bioassay, *Blattella germanica*

INTRODUCTION

Frass-contaminated shelter paper of *Blattella germanica* (L.) attracts and arrests insects on it. The phenomenon was believed to be initiated by a single behaviour modifying chemical named ‘aggregation pheromone’ (ISHII and KUWAHARA, 1967). We recognized, however, that attraction and arrestment were the consequences of different behavioural processes, so we established specific assay systems for each. First we focused on the attraction and designed an olfactometer for evaluating chemotaxis and odour-modulated anemotaxis of the insect (SAKUMA and FUKAMI, 1985). The identified attractant comprised ammonia and several alkylamines and aminoalcohols found as their hydrochloride (SAKUMA and FUKAMI, 1990b). If these chemicals serve both as attractants and arrestants, they should also test positive in Ishii’s paper assay (ISHII, 1970), which allows the insect to contact the material and settle on it. Starting with the verification of the arrestant activity of attractant amine salts, this article deals

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with the improvement of the arrestant assay, including the demonstration of the presence of the arrestant as well as the sense organ and the site of production.

MATERIALS AND METHODS

Animals. *Blattella germanica* (L.) were reared on insect diets (Oriental Yeast Co., Tokyo) at $29 \pm 1^\circ\text{C}$, $50 \pm 10\%$ RH and on a 14L–10D regime (SAKUMA and FUKAMI, 1985). Test animals were generally the mixture of first and second instar nymphs isolated from the colony at one to six days post-eclosion. Antenn- and/or maxillary palpectomized male adults were subjected to investigation of the sense organ. They were isolated from the colony, and after being immobilized at 5°C , treated with iridectomy scissors under microscopic observation.

Vertical paper assay and statistics. Vertical paper assays of amine hydrochlorides were conducted under almost the same protocol as ISHII's original method (ISHII, 1970). A pair of sample and control filter papers (Whatman No. 1, 2×8 cm, folded in W-shape) stood contiguously in the centre of a polyvinylchloride cup (8 ID $\times$ 4.5 cm). Inner walls of the cup had been dusted with talcum powder to confine the test animals within it. Twenty cups were prepared and some 10 to 30 nymphs were scattered in each cup. After 40 min, nymphs on each paper were counted and compared, while those on the bottom were neglected. If more nymphs were observed on the sample paper, the cup was denoted as '+', inversely '-'. The numbers of '+' and '-' cups were then compared; cups with the same number of nymphs on each paper were not counted. If significant difference was not observed by sign test ($p > 0.05$) (GOLDSTEIN, 1964), the arrestant activity was decided as ' $\pm$ ' (inert). If significantly more '+' cups were observed, the activity was decided as + (positive) (inversely as, -; negative). Assays were conducted during the day, at $25 \pm 0.1^\circ\text{C}$, $50 \pm 6\%$ RH, under 200 lux of fluorescent light illumination.

Amine hydrochlorides. Hydrochlorides of ammonia, methylamine, dimethylamine, trimethylamine, and 1-dimethylamino-2-methyl-2-propanol (1-DMAM-2-P) were subjected to the vertical paper assay. They were commercially available (Nacalai tesque, Kyoto) except 1-DMAM-2-P hydrochloride prepared in the laboratory. Each material (100 mmol) was dissolved in methanol or aqueous methanol (10 ml) and diluted decimally with methanol. Twenty pieces of the folded filter paper were piled and made to absorb 2 ml of each dilution in a small Petri dish. After air blown solvent evaporation, the treated papers were assayed. Control papers were treated with 2 ml of methanol.

Choice-chamber assay and statistics. Strips of sample and control paper (Whatman No. 1 or Toyo No. 2, 2.2×24.2 cm, 3 strips each) were aligned alternately on a glass plate ($15 \times 20 \times 0.2$ cm), folded at both ends, and fixed with sticky tape onto the back side of the plate. A polystyrene grating ($11 \times 18 \times 2$ cm, divided in 5×8 cells; inner frame of freezing container FC-2, Nichiden-Rika Glass Co. Ltd., Kobe) was placed on the plate, so that the floor of each cell (2×2 cm) was divided into sample and control areas in the centre (Fig. 1). The grating had been plastered with talcum powder to confine the test animal on the floor. One nymph was transferred from an applicator to each cell and then the grating was covered with another glass plate and a card of paper (white fine paper 128 g/m^2 , 12.8×18.2 cm, Kokuyo Co. Ltd., Osaka). The assembly was fixed with rubber bands and set on a level table. Nymphs on the sample

Fig. 1. Choice-chamber assay of *B. germanica* nymphs. Choice-chambers at 5, 20, and 40 min from the start. Nymphs significantly preferred the frass-contaminated paper (FCP) to clean paper (CLP) within 20 min, and the arrestment almost reached the maximum at 40 min.

area were counted with the shifting of paper at appropriate times (Fig. 1). Those lying across the border were counted according to head position. One assembly produced 40 subjects, and the response of five replications were totaled ($N=200$). In some experiments, response was observed every 15 min after 30 min from the start, and five observations were totaled ($N=200$). The arrestment activity was expressed in terms of excess proportion index (I), i.e. proportion of the difference between numbers of nymphs in sample and control areas to the sum (SAKUMA and FUKAMI, 1985). The assay conditions were the same as those of the vertical paper assay.

Assay for the antenn- and/or palpectomized male adults were conducted with almost the same conditions and set-up except for the paper strips (3.6×22.2 cm, 2 strips each) and grating (3×5 cells, 3.3×3.3 cm each).

Frass-contaminated paper. Sheets of filter paper (Toyo No. 2) were used as shelters in the mass culture (SAKUMA and FUKAMI, 1985, 1990a, 1990b). They were contaminated with excrement for six weeks. After frass particles on the surface were removed by brushing, the paper was cut into strips and then subjected to choice-chamber assay.

Evaluation of colour tone and darkness. Illuminance was measured by a Minolta digital luxmeter model T-1 (Minolta Camera Co. Ltd., Osaka). Colour tone of the paper was expressed as reflectance, which was evaluated by dividing illuminance of catopric light by that of the incident light.

Extraction of male adult body. Fifty male adults, having been anaesthetized with carbon dioxide gas and then with ethylether, were treated immediately. Each body was hung from the end of the abdomen with fine forceps and rinsed with methanol except for the anal opening. After the head and abdominal tip (9th and 10th segments) were dissected from the body, the abdomen was opened with irridectomy scissors and the alimentary tract with Malpighian tubules was extracted with forceps. The rectum and other parts of the abdominal tip were dissected with two fine forceps. In another experiment with 10 bodies, the abdominal tips were further isolated into several parts under binocular microscopic observation. Each part was extracted with methanol and the decimal dilutions were applied on the paper strips. After hot air blown drying (65°C) for one hr with an AD-675 futon dryer (Mitsubishi Electric Co., Tokyo), the strips were subjected to choice-chamber assay with nymphs. A dose of the extracts was expressed as 'male adult equivalent' and probit analysis on a set of dose/response evaluated ED_{50} of each extract (SAKUMA and FUKAMI, 1990a).

RESULTS

Vertical paper assay of attractant amine salts

Each hydrochloride of ammonia, methylamine, dimethylamine, trimethylamine, and 1-DMAM-2-P was subjected to vertical paper assay. Dose levels were adopted with reference to their attractant activity and natural contents in the frass contaminated paper (SAKUMA and FUKAMI, 1990b). Table 1 shows that all the examined attractant amine salts represented no arrestant activity except for slightly positive data on 1 mmol/paper of methylamine hydrochloride.

Interpretation of vertical paper assay results with clean papers

Although the arrestant activity of the frass contaminated paper in vertical paper

Table 1. Vertical paper assay of the attractant amine hydrochlorides for the arrestment of *B. germanica* nymphs

Amine hydrochlorides	Doses ^a				
	10 ⁻¹	10 ⁰	10 ¹	10 ²	10 ³ μ mol/paper
Ammonia	—	$\pm^b$ (10+, 10-) ^c	$\pm$ (9+, 10-)	$\pm$ (9+, 10-)	$\pm$ (8+, 10-)
Methylamine	—	$\pm$ (9+, 10-)	$\pm$ (10+, 8-)	$\pm$ (13+, 7-)	$\pm$ (15+, 5-)
Dimethylamine	—	$\pm$ (10+, 9-)	$\pm$ (12+, 8-)	$\pm$ (13+, 5-)	$\pm$ (9+, 8-)
Trimethylamine	—	$\pm$ (9+, 9-)	$\pm$ (9+, 9-)	$\pm$ (10+, 8-)	$\pm$ (9+, 11-)
1-Dimethylamino-2-methyl-2-propanol	$\pm$ (5+, 14-)	$\pm$ (5+, 14-)	$\pm$ (12+, 7-)	$\pm$ (6+, 14-)	$\pm$ (7+, 12-)

^a Each methanol dilution of amine hydrochloride was applied on 20 sheets of 2 $\times$ 8 cm Whatman No. 1 filter paper. Control papers were treated with methanol.

^b Judgement of the arrestant activity. ' $\pm$ ' denotes the inert condition with no significant difference between the number of '+' and '-' cups by the sign test ($p < 0.05$, two-tailed). More '+' cups with significant difference gave '+' (positive), inversely '-' (negative).

^c The numbers of '+' and '-' cups. '+' sign denotes the case that more nymphs were observed on the sample paper than on the control; '-' is the inverse.

assays was apparent (ISHII and KUWAHARA, 1967; ISHII, 1970), significant difference between the numbers of resting nymphs was also recorded in the assay with a pair of clean papers. Table 2 shows an example modified from ISHII's original data (ISHII, 1970). The sign test between the numbers of nymphs on the marked and control filter papers indicated that significant difference ($p < 0.05$) was observed in 13 pairs of 20, i.e. aggregation on one side prevailed. In such a case, the arrestant activity of the paper should be based on the preference of a group rather than of individual nymphs.

Influence of illuminance and reflectance in choice-chamber assay

Colour tone of the frass-contaminated paper or shadow of the cell wall could affect the preference. We examined the preference between the black (6% reflectance) and

Table 2. Preference tests between two blank filter papers for the first instar nymphs of *Blattella germanica*^a

No.	No. of nymphs aggregated on filter paper		No. of nymphs crawling	Sign of cups ^b	Results of sign test for No. of nymphs ^c
	Marked (+)	Control (-)			
1	7	5	0	+	±
2	15	2	0	+	+
3	1	9	0	-	-
4	1	15	0	-	-
5	2	7	0	-	±
6	0	8	0	-	-
7	0	12	0	-	-
8	23	3	0	+	+
9	11	20	0	-	±
10	17	1	0	+	+
11	1	8	0	-	-
12	0	12	0	-	-
13	0	19	4	-	-
14	7	5	0	+	±
15	11	21	0	-	±
16	8	0	0	+	+
17	10	0	1	+	+
18	5	3	0	+	±
19	13	8	0	+	±
20	2	11	0	-	-
Total	134	169	5	± (9+, 11-) ^d	(5+, 7±, 8-) ^e

^a This table was modified from Table 1 in the original article (ISHII, 1970). In the original article, *F*-test estimated whether the frequency of '+' cups was significantly larger than 0.5: practically the same as sign test.

^b Refer to note 'c' for Table 1.

^c Results of the sign test ($p < 0.05$) between the number of nymphs on the marked (+) and control papers (-).

^d Result of the sign test ($p < 0.05$) between the number of '+' and '-' cups. Also refer to note 'b' for Table 1.

^e Significant difference ($p < 0.05$) was recorded in 13 of the 20 cups (65%).

white (66%) paper strips under 200 lux illuminance of fluorescent light. To exclude other factors, we covered the paper strips with a matted sheet of transparent polypropylene to prevent nymphs from direct contact with the paper. Figure 2 shows that a significant preference to the black side was observed after 10 min. The tone of the frass-contaminated paper (36%), however, did not influence the preference. Then the preference between the light (200 lux illuminance) and shadow (23 lux) on the white paper floor was examined. The shadow was made by covering the structure with black paper strips. A preference for the shadow was observed after 20 min.

Fig. 2. Influence of illuminance and reflectance in choice-chamber assay of *B. germanica* nymphs. Although a significant preference of the black to white paper strips (●) was observed, colour tone of the frass-contaminated paper (○) did not affect the preference. Nymphs preferred shadow to light (▨). Vertical bars indicate 95% fiducial interval ($N=200$).

Fig. 3. Change in preference to the frass-contaminated paper on choice-chamber assay of *B. germanica* nymphs. Preference to the frass-contaminated paper (●) reached the maximal plateau at 10 min and was still observed at 12 hr. Covering with a sheet of gauze hindered the direct contact to the paper and the preference (○) was not recorded after 20 min. Vertical bars indicate 95% fiducial interval ($N=200$).

Arrestant activity of the frass-contaminated paper in choice-chamber assay

The frass-contaminated paper was subjected to choice-chamber assay. Figure 1 shows the choice-chambers at 5, 20, and 40 min from the start. Although a slight bias toward the frass-contaminated paper was observed, most of the nymphs were still walking at 5 min. The arrestment of nymphs increased at 20 min, and reached a maximum at 40 min. Closed circles in Fig. 3 indicate the result of the same experiment under a paper-shade (28 lux) without camera flashes. The response reached the maximal plateau at 10 min and maintained its level until 80 min. Arrestant activity was still observed at 12 hr, but the response decreased significantly. In order to examine the participated modality, we covered the paper strips with a sheet of fine polyester gauze (Tetoace #9000, Toray, Tokyo) permitting only the emanation of odour. Open circles in Fig. 3 represent the response of nymphs being hindered from direct contact with the paper. Although a slight bias toward the frass-contaminated paper was recorded up to 10 min, the response decreased and no preference was observed again.

Sense organ of the arrestant

We subjected male adults to amputation of both antennae and palpi and then subjected them to choice chamber assay (Fig. 4). They showed no preference toward the frass-contaminated paper. However, the treated adults retaining a pair of antennae or palpi responded to the arrestant almost equally to untreated individuals. Responses decreased by additional unilateral ectomy, but still remained positive. The distribution of the antennal receptors was surveyed by stepwise amputation of flagelli from bilaterally palpectomized male adults at each of pedicellus-flagellum joint, 20, 30, 40, and 50th

Fig. 4. Responsiveness of antenn- and/or palpectomized *B. germanica* male adults to the frass-contaminated paper. Male adults were treated as in the figure and subjected to choice-chamber assay with the frass-contaminated paper. Each pair of long rods and short spoons, attached to an oval head, illustrate antennae and maxillary palpi. Figure above each partially antennectomized head indicate the number of remaining flagellum annuli. Responses were expressed as excess proportion index with 95% fiducial limits in parenthesis.

Fig. 5. Arrestant activity of the extract from each body part of *B. germanica* male adults. Male adult bodies were dissected as in the figure and each part was extracted with methanol. The activity was expressed as ED₅₀ values in 'male adult equivalent/160 cm² of Whatman No. 1 filter paper'. Solid figures indicate positive response, whereas outlined figures are those for negative responses, *i.e.* preference to the control clean paper. Activity was localized in the supra-anal plate and genitalia.

annuli. Animals with 40 and 50 flagellum annuli responded, but those with 20 and 30 annuli did not.

Site of the arrestant production

Male adult bodies were dissected in parts and the methanol extracts were subjected to choice chamber assay. Figure 5 shows the anatomical scheme and arrestant activity of each extract (also refer to materials and methods section). The ED₅₀ values in 'male adult equivalent' indicate that the extract of abdominal tips of 9th and 10th segments exerted potent arrestant activity. The rectum extract, isolated from the abdominal tips, was almost inert, as were alimentary tracts. Rinse of the body was slightly active, but the rest of the body including thorax, legs, wings, and abdomen without alimentary tract exerted a negative response, *i.e.* preference to the control clean paper. The head extract exerted a negative response as well.

The abdominal tips were separated into dorsal and ventral parts. The dorsal part was dissected into rectum, the centre and peripheral parts of supra-anal plate (tergum 10; Fig. 6). The ventral part was separated into genitalia and integuments including subgenital plate (sternum 9). The arrestant activity was concentrated in the extract of genitalia and both extracts of supra-anal plate. As for the extracts of supra-anal plate, the centre part with anal accessories was significantly more active than the

Fig. 6. Ventral view of rectum (R), supra-anal plate (SAP), and cerci (C) of a *B. germanica* male adult. The dissection was conducted along the dotted lines so that the supra-anal plate was separated into the centre and peripheral parts. A projection (P) stuffed with fat bodies was found in each side of anus (A).

peripheral part with cerci. Again we could not find the arrestant activity with the rectum extract.

DISCUSSION

The attractant components of the *B. germanica* aggregation pheromone were identified as ammonia, several alkylamines, and aminoalcohols through olfactometer assay (SAKUMA and FUKAMI, 1990b). They are originally so volatile as to be unable to remain on the substrate for a long time, but in the natural condition they formed salt with hydrochloric acid and evaporated slowly from the frass-contaminated paper (unpublished). This slow release of the attractant amines provided another possibility that they were functioning as arrestants as well. We examined hydrochlorides of the principal attractant amines by vertical paper assay. In spite of the almost complete attraction in the olfactometer, all the examined attractant amines exerted no response in vertical paper assay. This contrasted with the concrete observations on the aggregation of *B. germanica* nymphs on the frass-contaminated paper (ISHII and KUWAHARA, 1967, 1968; ISHII, 1970; BELL et al., 1972), so that the presence of an arrestant chemical(s) other than the attractant amines was suggested.

In order to complete the assay system, we verified the statistics. The assay results were evaluated by comparing the numbers of nymphs on both sample and control papers. If each of the nymphs choose a paper independently, the arrestant activity would be directly reflected on the numbers of nymphs, so it may be sufficient to evaluate

the activity simply by counting great numbers of nymphs on a pair of papers. Results on pairs of clean papers showed, however, that they did not choose independently but related to each other and aggregated on one of the papers. In such cases, the number of individual nymphs does not precisely reflect the activity. Results with the same or similar assay system should be re-considered in this regard (ROTH and COHEN, 1973; TAKEDA, 1980; NAKAYAMA et al., 1984; ROSS and TIGNER, 1986; McFARLANE and ALLI, 1986). Consequently we dealt with a group of nymphs instead of individual nymphs. Whether the group gathered on the sample paper or not was approximately reflected by the numbers of nymphs. Therefore, after comparing the numbers on both papers, we put a plus or minus sign on each cup and judged the activity with sign test.

Although one will be able to obtain a quantitative result with a great number of paper pairs, it must be somewhat consumptive to prepare the set-up and some 20 test animals only to perform a single trial of the binomial system. We simplified the assay so that only the chemical factor might be reflected in the results. In the improved system, a single test animal on a level floor was made to choose between the sample and control area. This type would be categorized in choice chamber or alternative chamber, having been widely used for preference assays (KENNEDY, 1977, 1978). Our system provided arrays of cells which were easily prepared by combining paper strips and a grating. This made it possible to produce enough subjects for evaluating ED₅₀ with far less test animals, sample amount, and efforts than those of vertical paper assay.

In each cell of the choice-chamber apparatus, physical directional cues such as air movement and levelness of the floor were generally controlled except for light. It is conceivable that the nymphs might be arrested by a dark tone of frass-contaminated paper or by shadow of the cell wall. When an extreme contrast was produced between black and white, nymphs preferred black, but the tone of frass-contaminated paper was not sufficient to exert scototactic behaviour. Shadow did, however, affect the results to the same extent as the black paper did. Although *B. germanica* was believed to behave unaffectedly by light (CORNWELL, 1968), significant preference to the shadow was recorded. This factor could be excluded by indirect illumination through a sheet of slightly translucent paper covering the apparatus.

B. germanica nymphs were arrested by the frass-contaminated paper and generally became motionless within several min. The photograph in Fig. 1 shows that 80% of the nymphs were arrested at 20 min in spite of disturbance by occasional camera flashes. Once they settled, the response maintained for over 80 min and slowly decreased, probably because of self-recontamination and habituation to the stimuli. As the frass particles were carefully removed from the paper, the surface texture of the sample paper was not so different from the control. The influence of the colour tone was practically ruled out in the discussion. As a result, we could infer that the choice-chamber assay detected the arrestant activity just as well as vertical paper assay did.

The arrestment could be induced by both odour and contact chemicals. The results on gauze-covered papers showed that the nymphs significantly preferred the sample side in the first 10 min, but the response was not stable enough to last after 20 min. It is most likely that the attractant amines chemotactically attracted the nymphs on the frass-contaminated paper strips, but it is also possible that the amines or other odorous chemical(s) induced kinesis components which lead to the temporary arrest. The decline of the response could be attributed to the dispersion and homogenization of the odour vapor in a small cell and/or habituation of nymphs to the odour.

This odour-induced temporary arrest was not the result of inverse orthokinesis but of klinokinetic and klinotactic components, for the nymphs still moved about at 40 min. Inverse orthokinesis (the cessation of the movement) was observed when the nymphs were allowed to contact the paper directly. Thus it was suggested that the stable arrest was the result of inverse orthokinesis and this response was induced by non-volatile chemical being received through contact chemo-reception. This contact chemical-induced stable arrestment alone was detectable, after the odour-induced temporary arrestment waned, 20 min from the start (Fig. 3). A similar system can be applied to *Periplaneta americana* (L.) whose locomotion was effectively inhibited by direct contact to the frass-contaminated paper (BURK and BELL, 1973).

Since the arrestant was suggested to be a contact chemical, the candidate sense organs were antennae, maxillary palpi, labial palpi and tarsi. Labial palpi and tarsi were, however, ruled out, for the amputation of both antennae and maxillary palpi completely reduced the responsiveness. The animal with either a pair of antennae or maxillary palpi responded at almost the same level as the intact one. This means that the arrestant was received by both antennae and maxillary palpi. This bimodal reception contrasted with that of the attractant components solely by antennae (SAKUMA and FUKAMI, 1985; BLOCK and BELL, 1974 for *P. americana*). Moreover the animal with single antenna or palpus responded, suggesting that the single sense organ was enough to exert inverse orthokinesis. The antennal receptors seemed to be distributed between the 30–40th annuli and tip.

The site of the arrestant production was verified in expectation of an effective sampling. The aggregation pheromone of *B. germanica*, currently the arrestant, had been believed to be produced from the abdominal tip, in particular rectal pad cells of the rectum (ISHII and KUWAHARA, 1967). We dissected and extracted parts of male adult bodies accordingly. The potencies of the extracts indicated that the arrestant was produced from the abdominal tip region, but unexpectedly the rectum extract did not exert any activity. Five replications certified the result. Then we investigated the abdominal tip and tried to identify the production organ. The activity was found both in genitalia and the centre part of the supra-anal plate. As the arrestant was produced by the animal irrespective of sex and instar, it is hardly conceivable that male genitalia secretes it. The arrestant was probably secreted from the anal part and was adsorbed on genitalia usually contacting the anus. On each side of the anus, we could find a projection stuffed with fat bodies representing a glandular nature, but whether they produce the arrestant or not should be investigated in future work.

We adopted choice chamber assay simply because the arrestant activity was effectively evaluated, although this method is notorious for its ambiguity in discriminating behavioural components (KENNEDY, 1977). It allows test animals not only the direct contact with the material but also reciprocal choice between the sample and control area. These characteristics cause a systematic ambiguity, i.e. kinesis and taxis combine together. Moreover, at least two reception modals are concerned, i.e. odour and contact chemo-reception. These ambiguities, however, provide room for detecting the 'arrestant activity', which is intrinsically a mixture of several behavioural components. KENNEDY (1978) pointed out the importance of initiation and cessation of the stimulus and recommended 'temporally changing chamber' instead of choice chamber. His idea was applied on change in humidity (HAVUKKALA and KENNEDY, 1984) or odorous chemical (SABELIS et al., 1984), but how do we change temporally the amount of a

contact chemical? Fortunately the present assay system practically evaluates the activity of the contact chemical alone, so isolation becomes possible. The purification is now in progress and the behavioural analysis will follow it.

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