Spatial orientation of the mould mite, *Tyrophagus putrescentiae* (Schrank) (*Acarina: Acaridae*), in the computer-programmed olfactory field

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ABSTRACT

The attractiveness of food odor to the mould mite was demonstrated in the micro-locomotion compensator (MLC) as well as in a classical choice-chamber. The MLC has been developed for use in experiments on the orientation mechanisms of tiny animals. The positioning system of the MLC constantly returns a mite walking on a glass plate to the center of the apparatus by sliding the plate. A computer controlling the MLC logs the displacement of the plate, whose array defines the path of the mite. It also controls a micro solenoid-operated valve to generate odor cues in airflow over the mite. If the odor is generated by position in a virtual olfactory field created by the MLC, the mite would exert a similar olfactory response as in a real choice-chamber. In practice, odorous zones with attractant were defined on the glass plate in a checkered pattern, and the test animal was exposed to the odor while it stayed in the zones. Mites were effectively confined to the odorous zones in the virtual olfactory field, as well as in the real choice-chamber. The frequency of stays in the odorous zones positively correlated with the odor concentration to provide a reliable estimate of attractiveness using probit analysis.

Key words: attractant, locomotion compensator, mite, orientation, probit, virtual reality

INTRODUCTION

Animals exploit external information to modulate their behavior when searching for resources. This is also true of tiny astigmatid mites, including the mould mite *Tyrophagus putrescentiae* (Schrank), which track food resources by using olfactory cues. Several attractants have been isolated from the food materials (Yoshizawa et al., 1970; Vanhaelen et al., 1978; Sato et al., 1993). The attractiveness of these chemicals to mites has been demonstrated mainly by conventional choice chambers and equivalent assay methods, where the attractiveness was evaluated as the biased distribution of mites to treatment area from the control (Dethier, 1947; Kennedy, 1977). In these types of assays, however, mites freely cross an odor concentration gradient in stationary air, and exposure to the attractant odor is hardly controllable.

To overcome this problem, we have developed a micro-locomotion compensator (MLC) that is specially designed to compensate for every locomotive movement of an ambulatory animal of submillimeter size. The animal walks freely on a glass plate placed on a micro-
positioning apparatus. A feedback system continually slides the plate so that the test animal is kept at the same spatial position, thus cues can be presented in a precisely controlled manner. If the MLC is combined with an odor-generator, various models of the virtual olfactory field can be created (Sakuma, 2002). In the present study, odor was generated while a mite stayed in the computer-programmed ‘odorous zones’ on the plate.

This paper deals with the olfactory attraction to mites demonstrated in a virtual sensory field, as well as in a real choice-chamber.

MATERIALS AND METHODS

Mites. The mites were fed on dry yeast (Japan Pharmacopoeial, Asahi Food & Healthcare Ltd., Tokyo) at 25 °C and 70–80% relative humidity in a polystyrene culture dish (90 i.d. ×18 mm). They were starved for 24 h before experiments.

Food attractant. A methanolic extract of the dry yeast was used as an attractant in the choice-chamber assay and MLC experiments. An amount of 5–50,000 µg yeast equivalent (y.e.) was applied to dispensers in the experiments.

Choice-chamber assay. Sample solution (10 µl of methanol) was applied to a filter paper dispenser (5 mm square of #2; Advantec, Tokyo) and then air-dried. The treatment and solvent control dispensers, 10 mm apart, were elevated 15 mm from the bottom of a polystyrene culture dish (90 i.d.×18 mm). The lid of another dish, having been used for rearing mites, was gently placed on the dish (Fig. 1). The mites on the ceiling of the lid were not allowed to make direct contact with the dispenser. The number of mites on the ceiling surrounding each dispenser within 7.5 mm of the center of the dispenser was recorded after 5 min.

Micro Locomotion Compensator (MLC). The MLC comprises a positioning system, a video camera with a microscopic lens, a video tracker and a computer system (Fig. 2). The positioning system has a pair of motorized slides (SPF86B10-8P, Oriental motor Co. Ltd.,

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Fig. 1. Choice-chamber assay: Treatment and control filter paper dispensers (5 mm square) are elevated from the bottom of a culture dish (90 i.d.×18 mm), so that the distance between the dispensers and the ceiling of the lid is kept at 3 mm. Since mites walking on the ceiling are not allowed to make direct contact with the dispensers, only the olfactory response can be observed.
Tokyo) connected orthogonally each other, and the top table of the slides can be positioned with a precision of just micrometer. A mite freely walking on a glass plate on the table top is observed by the video camera, and after image-processing at the video-tracker (G-120, OKK Co. Ltd., Tokyo), the position of the mite is reported to a personal computer (IBM-PC compatible) at 60 Hz. The PC controls the pair of slides to return the mite to the previous position, so the mite is maintained at a point just under the video camera lens in the center of the apparatus. The PC also logs the displacement of the slides, whose array defines the path of the mite. The control software for this system is written in the C language and runs under the PC-DOS 7.0 (IBM Japan, Tokyo) operating system. The acquired data are analyzed by a second program, which runs under the Windows (Microsoft, Japan) operating system. Both programs were developed by the author (Sakuma, 2002). Details of the system will be reported elsewhere.

**Odor generator.** The attractant odor was provided by the odor generator (Fig. 3). The airflow originating at a compressor was adjusted to a flow rate of 20 ml/min and re-moistened to 85% r.h. A miniature solenoid-controlled valve just before the inlet to a cylindrical test chamber (5.0 i.d.×5 mm, ca. 100 µl vol.) switches the flow between odorous and odorless ducts of micro-pipette tips (0.5–200 µl vol., Asahi Techno Glass Corp., Tokyo). The sample solution and solvent methanol (10 µl of methanol) were applied to filter paper dispensers (5 mm square of #2; Advantec, Tokyo) and air-dried. They were then inserted into the odorous and odorless tips, respectively. The tips were plugged into the upper part of the chamber through ports on the wall, so when the chamber was covered with a piece of cover glass, the air from the tips moved downwards into the chamber.

**Virtual olfactory field.** The generation of odor was controlled by the spatial position of
The main program computes the position every 100 ms, and controls presentation of the odor cue by switching the solenoid-controlled valve. At the beginning of the experiment, odorous and odorless zones, being arranged in checkers, were defined on the glass plate arena. During the experiment, odor was generated while a mite stayed in the odorous zone. The track of a mite in the olfactory field can be observed in real time, and analyzed for the frequency of stays in both odorous and odorless zones, afterwards.

**Statistics.** Attractiveness recorded in the choice-chamber is expressed in terms of an excess proportion index (EPI): proportion of the difference between the number of mites surrounding the treatment dispenser ($N_t$) and that of mites surrounding the control dispenser ($N_c$) or

$$EPI = \frac{(N_t - N_c)}{(N_t + N_c)}$$

(Sakuma and Fukami, 1985). The EPI values range between −1 (complete repulsion) and +1 (complete attraction), and represent a neutral response at 0. The dose or amount of yeast extract is expressed as g y.e. (gram yeast equivalent) on each dispenser. The dose/response correlations in the preference results of choice-chamber assay and virtual olfactory field experiments were analyzed with a PriProbit program (Sakuma, 1998) to evaluate the attractiveness as median effective dose ($ED_{50}$). The EPI values and the $ED_{50}$ value for a dose
group were calculated also for the frequency of mites' stays in odorous and odorless zones in the olfactory field.

RESULTS AND DISCUSSION

The present study aimed to create a virtual sensory field that attracts a mite by evoking similar olfactory behavior to a real choice-chamber. If the sensory cues are presented to the mite in exactly the way as would occur during actual maneuvering towards a natural odor source, the mite will be attracted to a programmed goal or specific area in the field. The experiments in the virtual field, as well as those in a choice-chamber, were dedicated to the olfactory cue, and excluded directional components of mechanical and visual cues.

Mites in many conventional choice-chambers are allowed to make contact directly with sample materials (Yoshizawa et al., 1970; Vanhaelen et al., 1978; Sato et al., 1993). In the present choice-chamber system, mites walking on the ceiling of the lid are prevented from coming into direct contact with the odor-dispenser placed 3 mm below, and are only exposed to the odor emanating from the dispenser. This arrangement of the test arena makes it possible to discriminate the olfactory cue from the contact one.

The mites aggregated over the treatment dispensers even without direct contact (Fig. 4). Table 1 shows that they were significantly attracted to the dry yeast extract at least at 500 µg yeast equivalent (y.e.). The numbers of mites surrounding the treatment and control dispensers at a graded series of doses were subjected to the probit analysis for preference data (Sakuma, 1998). The procedure temporarily evaluated the $ED_{50}$ with 95% limits as
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Table 1. The attractiveness of dry yeast extract to *Tyrophagus putrescentiae* in a choice-chamber assay

<table>
<thead>
<tr>
<th>Doses(^a) ((\mu g) y.e.)</th>
<th>Number of mites around the dispenser(^b)</th>
<th>EPI (95% limits)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Control</td>
</tr>
<tr>
<td>0.0</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>5.0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>500</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>5000</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td>50,000</td>
<td>25</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\) g y.e.: Gram yeast equivalent of the methanolic extract of dry yeast applied to a filter paper dispenser (5 mm square).

\(^b\) The number of mites on the ceiling of a culture dish that surround each dispenser within 7.5 mm of its center. Also refer to the legend for Fig. 5.

\(^c\) EPI: The excess proportion index, i.e., the excess proportion of the number of mites around the treatment dispenser (\(Nt\)) to that of mites surrounding the control dispenser (\(Nc\)), or \(EPI = (Nt−Nc)/(Nt+Nc)\). Fiducial limits were calculated from the variance ratio, \(F\).

\(^d\) Regression formula obtained by probit analysis for preference data. \(Y\): Normal equivalent deviate, \(X\): Log (dose). Significance was examined by a \(\chi^2\) test on heterogeneity.

\(^e\) \(ED_{50}\): Median effective dose. A normal deviate was used in calculating the limits.

0.961 (0.00479, 7.30) mg y.e., but the fiducial interval was too extended for a reliable estimate. This may be partly because of the scanty numbers of subjects in lower dose groups, which totally depend on the random dispersal of mites. Since there is no movement of air, except for a small convective circulation, to evoke an upwind orientation, the only cue that mites can employ in this condition is the odor concentration gradient emerging from the treatment dispenser. In addition, mites would perceive the gradient as a temporally changing olfactory stimulus, for they are unlikely to compare odor intensity bilaterally.

This sensory environment can be simulated by switching automatically the odorous and odorless air-flows moving down-streaming onto the mite based on its spatial position. Such a programmed cue presentation in real time may provide a more realistic representation of the environment that mites perceive. This 'virtual reality experiment' employing a locomotion compensator was advocated by one of the authors (Sakuma, 2002).

In this study, a newly developed micro-locomotion compensator (MLC) with motorized slides was used instead of the servosphere apparatus employed in the preceding paper. This is because the minute displacement of a mite requires precise positioning at a level of micrometers, and the clearance between the floor surface and test chamber should be maintained within a marginal value. Both requirements are fulfilled successfully by the MLC.

The odorous zones were arranged in a checkered pattern whose size is changeable by the main program. The program computes the position of the mite every 100 ms, and controls
presentation of the odor by switching a solenoid-controlled valve. At the beginning of the experiment, odorous and odorless zones, being arranged in checkers, were defined on the glass plate arena. During the experiment, odor was generated while the mite stayed in the odorous zone. The track of the mite in the virtual olfactory field was observed in real time, and later analyzed for frequency of stays in both odorous and odorless zones.

Since the odorous zones were highly standardized and dynamically controlled, the attractiveness of a chemical could be evaluated quantitatively. Table 2 shows that the mites stayed in the odorous zones more frequently as dose increased. The correlation between dose and frequency of stays was analyzed with the probit regression. Although the assay system was different, the \( ED_{50} \) at 1.68 (0.853, 3.49) mg y.e./dispenser in the virtual olfactory field was the same level as that in the choice-chamber assay. Moreover, its fiducial interval was narrow enough for the estimate to be a reliable one. This must be due to the standardized cue presentation, and also to the sufficient number of subjects, being based on the frequency of stays, irrespective of dose levels.

Table 2. The attractiveness of dry yeast extract to Tyrophagus putrescentiae in a virtual olfactory field created by a micro-locomotion compensator system.

<table>
<thead>
<tr>
<th>Doses(^a) (µg y.e.)</th>
<th>Frequency of stays in(^b)</th>
<th>EPI (95% limits)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odorous zones</td>
<td>Odorless zones</td>
</tr>
<tr>
<td>0.0</td>
<td>110</td>
<td>90</td>
</tr>
<tr>
<td>50</td>
<td>117</td>
<td>93</td>
</tr>
<tr>
<td>500</td>
<td>129</td>
<td>71</td>
</tr>
<tr>
<td>5000</td>
<td>166</td>
<td>34</td>
</tr>
</tbody>
</table>

Regression Formula \( (x^2 \text{ test}) \)

\[ Y = -2.51089 + 0.77822 X \ (p>0.05) \]

\( ED_{50} \) (95% limits)\(^d\) (µg y.e.)

1680 (853, 3490)

\(^a\) g y.e.: Gram yeast equivalent of the methanolic extract of dry yeast applied to a filter paper dispenser (5 mm square).

\(^b\) The positions of the mite were recorded every 6 s for 5 min. Four replications showing no significant differences in potency and parallelism were pooled. Also refer to Table 1.

Since the odorous zones were highly standardized and dynamically controlled, the attractiveness of a chemical could be evaluated quantitatively. Table 2 shows that the mites stayed in the odorous zones more frequently as dose increased. The correlation between dose and frequency of stays was analyzed with the probit regression. Although the assay system was different, the \( ED_{50} \) at 1.68 (0.853, 3.49) mg y.e./dispenser in the virtual olfactory field was the same level as that in the choice-chamber assay. Moreover, its fiducial interval was narrow enough for the estimate to be a reliable one. This must be due to the standardized cue presentation, and also to the sufficient number of subjects, being based on the frequency of stays, irrespective of dose levels.

The experiments so far have demonstrated that the attraction of computer-programmed odorous zones functioned virtually the same as that in the choice-chamber assay. By minimizing the volume of the test chamber to 100 µl, the air supplied at 20 ml/min can be exchanged in 300 ms, and an almost instant change between odorous and odorless air can be achieved at the boundary of the zones. A mite walking in the virtual olfactory field should experience similar temporal changes of the odor cue as those in a choice-chamber, but the stimulus intensity would change more sharply than in a real space. This could cause a mite to exert behavioral processes leading to a longer stay in the odorous zones.

In this case, the existence of an odor-modulated upwind anemotaxis component (Kennedy, 1977; McMahon and Guerin, 2000) is ruled out, for the horizontally walking mites receive no directional cue from the vertically flowing air. The only cue that the mites could receive is the presence and absence, or the onset and cessation, of the olfactory
stimulus. Fig. 5 shows the tracks of mites concentrated in the odorous zones. When the mites stepped out of the odorous zones, they almost always made a large one-sided turn that led back into the odorous zones. In contrast, the mites entering the odorous zones seldom made a specific turn. Thus, it is inferred that the maintenance of turning angle at the cessa-
tion of the attractant odor caused mites to be attracted to the odor. This is a 'kinesis' response, for the animal could not refer to directional information, and used only the temporal change in the olfactory stimulus detected successively along its path (Kennedy, 1986). A more complete analysis of orientation behavior will be reported elsewhere.

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REFERENCES


摘 要

プログラムされた嗅覚空間におけるケナガコナダニの空間定位 小嶋 健, 佐久間正幸 *, 福井昌夫（京都大学大学院 農学研究科 応用生物科学専攻 昆虫生理学分野）、桑原保正（京都大学大学院 農学研究科 応用生命科学専攻 化学生態学分野）
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ケナガコナダニの食餌誘引物質の誘引性を、通常の選択室試験とさらに微小移動運動補償装置（MLC）で調べた。MLC は微小動物の定位機構を調べるために、新たに開発した装置である。MLC の位置決め装置はガラスの天板を滑らせて、その上を歩行するダニを常に装置の中心に引き戻す。MLC を制御するコンピューターは、ダニの軌跡となる天板の移動を記録
するとともに、電磁バルブを制御してダニに与える気流の匂い付けをおこなう。MLCが創る仮想空間内でのダニの位置に応じて匂い付けをおこなうならば、ダニは選択室と同様の嗅覚応答をするものと期待される。天板上に市松模様の区画を設け、ダニがその中にいるときにだけ匂いを与えたところ、選択室と同じく、ダニは匂い付けされた区画に効果的に留まった。匂い付けされた区画における滞在頻度は、匂いの濃度と正の相関を示し、プロビット分析の結果、誘引効果について精度の高い推定値が得られた。