BEHAVIOURAL AND ELECTROPHYSIOLOGICAL RESPONSES OF HOUSEFLIES TO ATTRACTIVE ODOURS

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Summary
Comparison of several natural products shows that houseflies are especially attracted to tainted meat and chicken manure. Males are also attracted to soaked bread products. GC-EAG was used to identify some electrophysiologically active chemicals from these products. No differences in sensitivity of olfactory cells between males and females were found.

INTRODUCTION

Houseflies (Musca domestica L.) are a nuisance to man and animals and are potential vectors of pathogens. One of the methods to control flies is using attractive odours to lure them to a trap (Cossé & Baker, 1996). However, existing commercially available baits showed variable results (Browne, 1990).

The most effective attractants for houseflies appear to be natural products, especially products of putrefaction (amines) and fermentation (alcohols, acids, aldehydes, ketones), which may serve as oviposition sites and food sources (Künstl & Günzrodt, 1981). It is known that manure and spilled food are the principal breeding media for houseflies. Dairy products and sugar-containing products (mono- and disaccharides) are also found to be attractive (Künstl & Günzrodt, 1981). It was shown that mixtures are more attractive than single chemicals (Brown et al., 1961; Mulla et al., 1977; Künstl & Günzrodt, 1981).

We try to identify the active components in attractive natural products in order to use these for increasing the effectiveness of baits for housefly control.

MATERIALS AND METHODS

Behavioural studies
The attractiveness of odours of several natural products was examined in a flight chamber illuminated by a white fluorescent lamp. Air (approx. 0.7 m/s) was led through two glass cylinders (i.d. 3.5 cm) positioned in plastic tubes (i.d. 8 cm) at the upwind end of the chamber. An odour source was placed in one of the cylinders behind a piece of gauze to prevent flies from contacting it. The other tube contained an empty cylinder. Initially, in each experiment 100-200 flies, males and females of one and the same age, were introduced
into the chamber. The flies had either been deprived of food during approximately 24 hours prior to the experiment or had been offered food until the beginning of the test ("well-fed"). The age of the flies ranged from 0-2 days (young) to 3-10 days (mature). During 20 minutes the number of landings in both tubes was noted. Later, experiments were done in a similar way with groups of males and females separately, all mature and well-fed. As a measure of attractiveness of an odour source the quotient of the number of flies that had landed in the odour-loaded tube and the total number of flies in the chamber was taken.

**Chemical analyses**

Extractions of natural products were made by stirring them in ethanol or hexane. The solution was filtered and separated in components with a Shimadzu GC-17A gas chromatograph, using a 15m x 0.32mm CP-Sil-5 CB column (Chrompack). The column was splitted and half of the effluent collected in a pasteur pipette. The pipette was replaced every minute and immediately used for EAG to check whether it contained electrophysiologically active components. Analyses of components were done with a GC-Mass Spectrometer (Hewlett-Packard), using a similar column.

**Electrophysiology**

An intact fly was immobilized in a Finn-pipette with its head protruding out of the tip. EAGs and activities of individual odour receptor cells were recorded with glass micropipette/Ag-AgCl electrodes filled with Ringer solution. Air was blown for 1s through a pasteur pipette loaded with GC-effluent into an airflow flowing over the antennae. EAGs were recorded using the technique described by Den Otter et al. (1988). For single cell recordings the surface-contact technique was used (Den Otter et al., 1980). Preparation of odour stimuli and analysis of responses were performed as described in Kelling & Den Otter (1998).

**RESULTS**

Initially, the odours of 24 natural products, such as manure, tainted meat, and fruits, were tested for their attractiveness. Figure 1 shows the mean responses of four categories of flies to the odours tested. The number of landings in de control tube was always very small. Both young and mature flies, either well-fed or food-deprived, were attracted to chicken manure, chicken meat, pork (9-11), and bread soaked in water or milk (23, 24). The average number of landings increased when the flies had been deprived of food during one day (Figures 1c and d). Food-deprived flies were also attracted to the odours of fruits (2, 3, 5, 6) (except lemon: 4) and yeast-containing products (16, 20, 21).

Figure 2 shows the responses of males and females separately to some odours found to be attractive in the previous experiments and to odours reported to be attractive in the literature. It appears that males were attracted to the odours of soaked bread (10-13), whereas these odours were hardly or not attractive to females. On the other hand, yeast, marmite, chicken meat and chicken manure (4, 5, 8, 9) attracted more females than males. Like in the previous experiments, only a few (well-fed) flies landed in response to the odours of fruit (2, 3).

The attractive products chicken manure and tainted pork were used for GC-EAG analysis to identify components that induce attractiveness. Figure 3 shows the chromatogram of an ethanol extract of chicken manure and EAGs obtained during stimulation with the GC effluents collected in the previous minute. Components eluted in the first 6 minutes of the run, being the most volatile components, elicited the highest EAGs. Using MS, some

Figure 3. Upper trace: Chromatogram of chicken manure extract. Lower trace: EAG signals of a housefly antenna. Each EAG-peak is the response to the compounds in the effluent of the previous minute except the first EAG, which is the response to the whole extract.
components eliciting EAGs could be identified. In chicken manure, these were acetic acid, 3-methylbutanoic acid, some phenoles, skatole, and indole. 3-methyl butanoic acid, phenol and indole were also present in tainted pork. The high peaks at 15-18 minutes (long fatty acids and their esters) did not induce large EAGs.

Dose-response curves for acetic acid, 3-methylphenol and skatole were made. Several olfactory cells show excitatory responses to acetic acid, 3-methylphenol and skatole (Figure 4a, c and d). The responses increased significantly with the increase of dose. Some cells that do not respond to acetic acid at the lower doses are inhibited by the highest dose (10 mg, Figure 4b). Repeated Measurements ANOVA showed no significant differences in dose-response curves between males and females (acetic acid: p=0.119; 3-methylphenol: p=0.385; skatole p=0.192).
DISCUSSION

Several natural substances were tested for their attractiveness for houseflies. Tainted meat (pork and chicken) and chicken manure were found to be the most attractive, whether the flies were young or mature, well-fed or deprived of food. Soaked bread attracted only males, whereas yeast, marmite, chicken meat and especially chicken manure were more attractive to females than to males. GC-EAG analyses were done with tainted pork (no difference in attractiveness between sexes) and chicken manure (large difference in attractiveness between sexes). These analyses indicated several components that were electrophysiologically active. Dose-response curves of some of the chemicals present in chicken manure (acetic acid, 3-methylphenol and skatole) were done to investigate any differences of sensitivity of olfactory cells between sexes. No significant differences were found. Therefore, based on these results, differences in behaviour to the same odours in males and females result presumably from higher-level decisions and not from receptor-level differences.

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REFERENCES


