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Short communications

Sensilla on cabbage root fly tarsae sensitive to egg-associated compounds

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Summary. We identified a tarsal sensillum that has a receptor neurone sensitive to a methanol extract of cabbage root fly eggs. This extract is known to act as an oviposition deterrent. The electrophysiologically active substance in the extract is probably not of host plant origin but a pheromone produced by adult flies.

Key words. *Delia radicum* (Diptera, Anthomyiidae) – cabbage root fly – oviposition – marking – pheromone – contact chemosensillum

Introduction

The cabbage root fly, *Delia radicum*, marks its host plant with an oviposition deterring substance (De Jong & Städler 2001), as do many other herbivorous flies (Prokopy 1981). Such markers discourage other females from ovipositing on the same host, thereby reducing the competition among the larvae for food. But De Jong & Städler (2001) also demonstrated that sand particles associated with the location of the eggs stimulate other females to oviposit, a phenomenon that may contribute to the clustered distribution of the eggs in the field (Harcourt 1967). Clustering of eggs may have advantages, such as increasing protection against desiccation and predation (McCall & Cameron 1995), and as a means of overcoming host resistance (Judd & Borden 1992). The combined information from marking on the plant that deters oviposition and marking on the sand that stimulates oviposition is thought to aid the flies in optimising the size of egg clusters in relation to the food available (De Jong & Städler 2001). The fact that methanol extracts of eggs applied on artificial leaves deterred oviposition would indicate that the cabbage root fly's host marker is associated with the eggs (De Jong & Städler 2001).

Relatively few marking pheromones have so far been isolated and chemically identified, despite their obvious importance. This is mainly due to difficulties encountered in collecting pheromones in amounts sufficient for purification and identification, as well as to logistic problems in screening the activity of fractions during the purification processes. Electrophysiological recording has proved to be extremely useful as a tool in such purification procedures (Städler *et al.* 1987; Hurter *et al.* 1999). Apart from the enormous time gains, compared to screening based on behavioural parameters, electrophysiology often requires far less material. The main challenge in electrophysiological testing of fractions lies in identifying the relevant sensory sensillum. Here we describe a tarsal taste sensillum that is sensitive to an extract of cabbage root fly eggs.

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Material and methods

Delia radicum flies were reared in climate-controlled rooms (21°C, 80% R.H., LD 16 : 8) following the method of Finch & Coaker (1969).

Electrophysiology. Recordings were obtained using standard tip recording techniques as described by De Jong *et al.* (2000).

Extracts. Egg extracts were made by carefully removing eggs from the sand substrate and storing them in methanol at 5°C for several days. The resulting methanol extract was evaporated to dryness and dissolved in 10 mM KCl for electrophysiology.

Insect extracts were made from freshly emerged (1-day-old) female (92) and male (102) flies, and from 7-day-old female (102) and male (71) flies, by grinding them in methanol. After 24-h in storage at 5°C, the solutions were filtered, evaporated to dryness and taken up in 10 mM KCl, for electrophysiological testing at concentrations ranging from 0.01 to 100 fly equivalents/ml.

Results and discussion

Electrophysiological recordings from tarsal sensilla on the front legs revealed that a pair of sensilla chaetica, located medially near the distal part of the 5th tarsomeres (*i.e.* "C" sensilla, according to Grabowsky & Dethier 1954), contain a receptor neurone that is sensitive to the egg extract (Fig. 1). These "C_5" sensilla have four chemoreceptor neurones (Isidoro *et al.* 1994), and are known to be sensitive to only a few other stimuli, *viz.* "CIF" (a group of compounds found in Brassica; Roessingh *et al.* 1997; Hurter *et al.* 1999; De Jong *et al.* 2000) and certain glucosinolates (Roessingh *et al.* 1992).

Since their only known stimuli are host specific compounds, C_5 sensilla are thought to be involved in the cabbage root fly's assessment of host plant suitability (Roessingh *et al.* 1997). In order to determine whether the active substance in the egg extract also originates from host plants and may have been sequestered during larval feeding, we tested extracts made

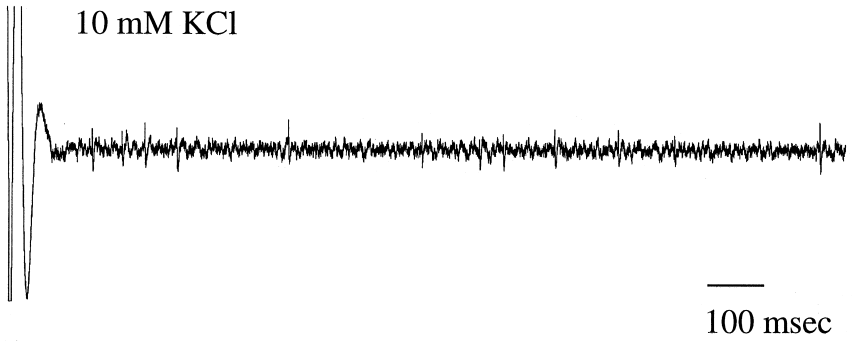
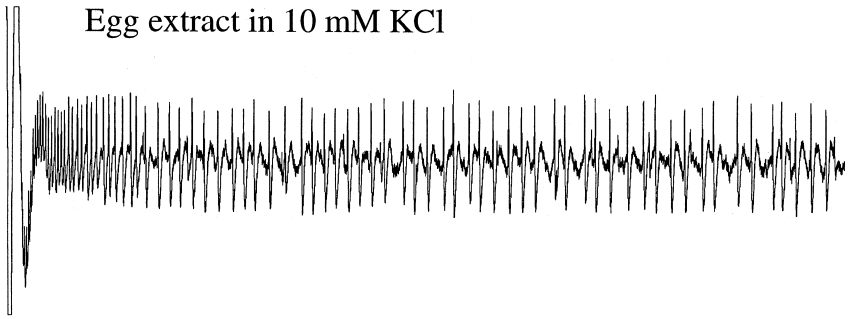
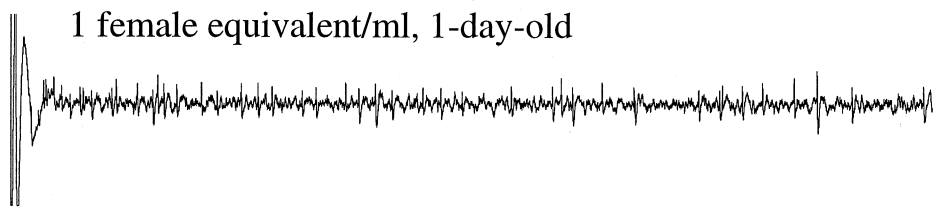
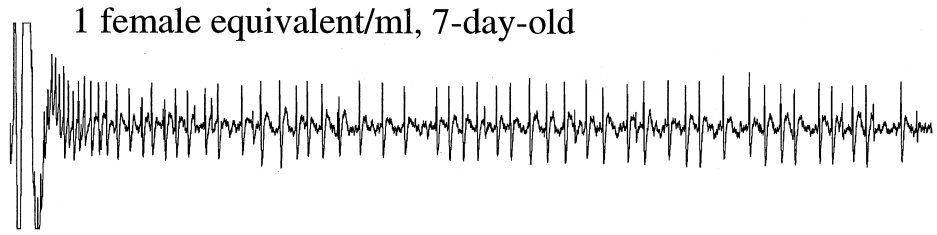
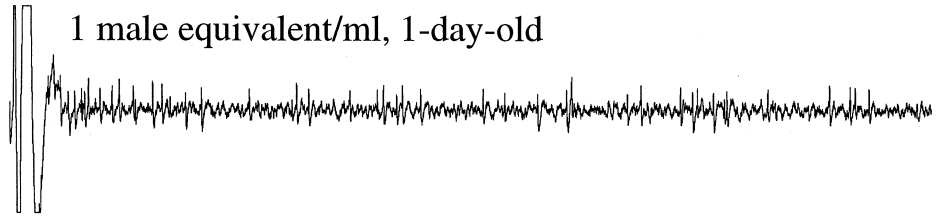


Fig. 1 Responses of a female cabbage root fly C_5 sensillum to stimulation with 10 mM KCl and a methanol extract of 0.01 g eggs in 10 mM KCl









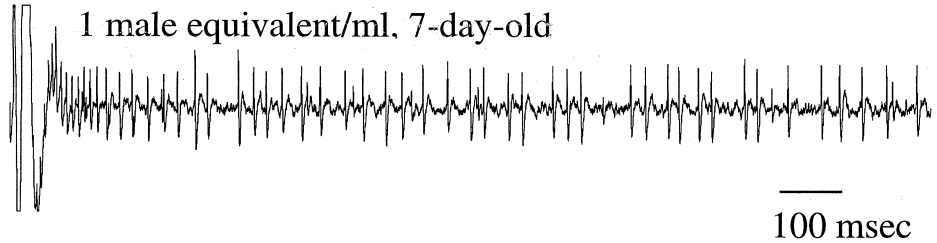


Fig. 2 Responses of a female cabbage root fly C_5 sensillum to stimulation with methanol extracts of 1-day-old female flies, 7-day-old female flies, 1-day-old male flies and 7-day-old male flies (all stimuli in 10 mM KCl)

from flies. The optimal concentration for electrophysiological testing of these extracts appeared to be 1 fly equivalent/ml. Higher concentrations were too viscous and blocked the recording electrode, which impaired recording. Extracts of 7-day-old female and male flies contain a substance that activates a receptor neurone in female C_5 sensilla (Fig. 2). Extracts of 1-day-old females and males were not stimulatory. This would indicate that very young flies lack this substance, and, consequently, that the active substance is produced during adult life.

The cabbage root fly's C_5 sensilla seem to be sensitive to only very few substances. However, it is not clear whether the activities elicited by the extracts of eggs and flies are due to the same material, and whether the oviposition deterrent effect is indeed caused by the electrophysiologically active fraction of the egg extract. If so, it would be interesting to know why a pheromone for marking host plants is associated to eggs. The identification of the contact chemosensillum that contains neurones sensitive to the egg extract should make it possible to purify the active substance, and might finally prove to be an important tool leading to the elucidation of the chemical structure of the cabbage root fly's oviposition marking pheromone. In comparable studies with the apple maggot, *Rhagoletis pomonella*, C_5 sensilla have been implicated in the detection of an oviposition deterrent pheromone (Crnjar & Prokopy 1982).

It is intriguing that an active substance is not only found in females. Males also produce a substance that activates female C_5 sensilla, and this may well be the same active component that is responsible for activity in the egg extract. Moreover, C_5 sensilla of male cabbage root flies are sensitive to the egg extract (unpublished results). The reasons for this are unclear, but one could speculate that the oviposition marking pheromone in the cabbage root fly has evolved from a pheromone used by both sexes, perhaps an aggregation pheromone. The results may indicate that this pheromone continues to be of importance to the males. In the European cherry fruit fly, *Rhagoletis cerasi*, males have receptors for the detection of female marking pheromone (Städler *et al.* 1994), and respond with arrestment after contacting it (Katsoyannos 1975). However, male European cherry fruit flies are not known to produce the same or a similar pheromone as the females (Städler *et al.* 1992), but male-marking does occur in several fly species, including the genus *Rhagoletis* (Papaj *et al.* 1996).

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