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## Pheromones of the housefly

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## SUMMARY

Houseflies (*Musca domestica* L.) often make themselves a nuisance in human and livestock habitations. Moreover, these flies may transmit over a hundred different pathogens. They can transmit intestinal worms or their eggs, and are potential vectors of pathogens of, for example, dysentery, gastroenteritis, typhoid, cholera and tuberculosis. Organic materials and the relative high temperatures present in animal and human dwellings promote rapid development and allow the continuous presence of the flies. The main goal of the studies described in this thesis was to reveal the role that certain semiochemicals produced by *M. domestica* could play in environmentally friendly control of the flies.

According to the literature of the last three decades, a pheromone is present on the body of housefly females which induces sexual behaviour in males. The main component of this female sex pheromone is supposed to be (Z)-9-tricosene ('muscalure'), one of the more than 150 cuticular hydrocarbons of females. (Z)-9-tricosene was found to be present in relatively high amounts on the females. This knowledge on the production of cuticular hydrocarbons and on their role in the behaviour of the flies has mainly been collected from laboratory strains of the flies. However, in order to be able to control the flies in their natural environments, we considered studies on wild-type flies also necessary. Therefore, we paid attention to houseflies which had been kept in culture in the laboratory for 40 years (WHO strain of flies), but also to flies obtained from a poultry breeding (Van Diermen strain) and from a cow-house with pig-sty (Pesse strain). The latter two strains had been cultured in the laboratory for different numbers of generations.

In Chapter 2 gas chromatographical studies are described with showed striking differences between the cuticular hydrocarbon composition of females of the WHO strain and females of the Van Diermen and Pesse strains. It appeared that on WHO females hydrocarbons with 23-25 C atoms constituted about 65% of the total hydrocarbons, whereas on first-generation wild-type females less than 2% of these compounds was present. Muscalure comprised up to 20-30% of the total hydrocarbons on 5-20-day-old WHO females, whereas less than 0.5% muscalure was present on the wild-type females. On males of both the WHO and wild-type strains (Z)-9-heptacosene was the most abundant hydrocarbon; males did not produce muscalure. It was found that in the course of time in 'mixed populations' (both sexes

in one and the same cage) muscalure was transferred from females to males and (Z)-9-heptacosene from males to females. It is suggested that this transfer of hydrocarbons between the sexes may result in modification of the original male and female cuticular hydrocarbon composition and may enable one sex to recognize whether the other sex has already copulated or not.

The marked differences in muscalure quantities on WHO and first-generation wild-type females led us to suggest that selection in subsequent generations of high-density populations may lead to increased production of muscalure by the females. Indeed, we found that after several generations in the laboratory the amounts of muscalure on females had increased considerably (Chapters 2 and 3). Apparently, higher amounts of this substance increased the attractiveness of females to males. Selection did not affect the production of other cuticular hydrocarbons by the females apart from that of tricosane, the production of which was shown to be closely linked to that of muscalure.

Despite the low quantities of muscalure on wild-type females no differences in reproduction capacity were observed between the different strains, which suggested that muscalure is not indispensable for mating. To investigate the role of muscalure in more detail we studied the effects of females from strains containing different muscalure quantities on sexual behaviour of the males. In addition, we determined the olfactory sensitivity of males and females from the different strains to muscalure by recording the electrical responses (electroantennograms) of their antennal olfactory cells (Chapter 3). The results showed that male sexual activity was higher towards females with higher amounts of muscalure on their cuticle. Moreover, males from strains with more muscalure on the females appeared to be more active, indicating that selection in laboratory cultures not only increases muscalure production in females but also sexual activity of males. The electroantennogram recordings indicated that males as well as females of all three strains were equally able to detect muscalure, which suggested that differences in sexual behaviour were not due to differences in ability to smell this substance.

Apart from acting as pheromones, cuticular hydrocarbons of insects provide a barrier to water diffusion, thus preventing desiccation of the animals. It is known that environmental factors may affect the production of cuticular hydrocarbons. As was remarked above, on females of subsequent generations of laboratory populations, cultured at constant temperature and humidity, the quantities of both muscalure and

tricosane increased, whereas those of the other cuticular hydrocarbons remained the same. This led us to assume that temperature and humidity may mainly determine the quantities of the non-pheromonal components, whereas muscalure may primarily be produced as a result of a selection process in high-density populations in isolated environments. We expected that selection proceeds faster in high-density than in low-density populations.

In Chapter 4 we studied the effects of relative humidity, temperature and population density on the production of cuticular hydrocarbons. The results showed that the production of these substances by both males and females of *M. domestica* was delayed up to at least 3 days after emergence under very wet conditions (90% r.h.) compared to the production at 50 and 20% r.h. Eight days after emergence, however, males contained the same amounts of hydrocarbons at all three r.h. values, whereas females still possessed less of these substances at 90% r.h. than at 50 and 20% r.h. In our opinion this was due to the fact that males, being more active than females, need more cuticular hydrocarbons to prevent water-loss than females. No indication was found that the r.h. has a different effect on the production of muscalure by females than on the production of the other hydrocarbons. As to the effects of temperature, it appeared that male and female flies produced more hydrocarbons at 35 °C than at 20 °C. In male flies the relative amounts of the various hydrocarbons produced at the two temperatures were the same. In females the production of muscalure at the two temperatures did not differ. However, the relative amounts of nonacosane and of the methyl- and dimethylnonacosanes were significantly higher at 35 °C than at 20 °C. We proposed that this had led to an increase of the melting temperature of the whole mixture of cuticular hydrocarbons, preventing extreme loss of water. Since the melting temperature of the whole mixture of hydrocarbons is higher in males than in females (39.4 and 36.8 °C, respectively: Gibbs *et al.*, 1995), we supposed that in males there was no need to change the composition of cuticular hydrocarbons when the temperature is raised to 35 °C. In low-density population cultures (< 20 flies/cage) all females of the 8<sup>th</sup> generation produced a low amount of muscalure, whereas no muscalure was found on the high-density (> 300 flies/cage) females. Hence, our hypothesis that the production of muscalure may not be affected by changes in humidity and temperature to the same extent as the production of other hydrocarbons did not hold true. The production of muscalure was affected in a similar way by these factors as that of the other hydrocarbons. The population-density

experiments showed that selection may sneak in very rapidly and should always be a point of major attention in laboratory colonies. We assume that because of the relatively large contribution to the total population the properties of a few females are likely to be expressed sooner in the next generations of small populations than in those of large populations.

Studies to reveal the role of cuticular semiochemicals on sexual behaviour of *M. domestica* often imply topical application of the chemicals to the flies when the chemicals are dissolved in an organic solvent like hexane or acetone. In Chapter 5 we showed that this way of application of semiochemicals may strongly affect the flies' condition. In addition, the distribution of these substances over the various body parts may deviate from the natural distribution. We showed that hydrocarbons, when liquid at room temperature, are also taken up and are distributed over the body in a more natural way when the flies are walking on a filter paper onto which the pure chemicals had been pipetted. The higher the amounts of hydrocarbons on the paper and the longer the flies walked on it, the higher the amounts of chemicals taken up.

Using this new "self-loading" technique, females were loaded with (Z)-9-heptacosene or (Z)-9-pentacosene. The former substance is the most abundant hydrocarbon on the cuticle of male houseflies and hardly occurs on females. (Z)-9-pentacosene is absent on houseflies, but acts as a female sex pheromone of the little housefly *Fannia canicularis*, which often is sympatric with *M. domestica*. We hypothesized that both (Z)-9-heptacosene and (Z)-9-pentacosene may inhibit sexual behaviour in male houseflies. However, we found that in contrast to this (Z)-9-heptacosene stimulated copulation when present in relatively high amounts on females, whereas (Z)-9-pentacosene did not affect male sexual behaviour.

In Chapter 6 a radar-Doppler actometer is described with which movements of individual body parts of the flies can be recorded. With this actometer movements of legs, wings and head could be distinguished. Discrimination between movements was possible by comparing the shapes and amplitudes of the recordings visualized in an actogram. Movements of the insect's head were used to obtain an indication of the behavioural responses of houseflies to (Z)-9-tricosene, (Z)-9-heneicosene and a 7:3 mixture of these two chemicals. A comparison was made with the results of EAG recordings obtained in a previous study (Chapter 3) on stimulation with the same substances. It was found that, although (Z)-9-heneicosene evoked high EAG responses in males as well as females, both sexes did not show behavioural responses to this

substance. Comparison of our results with those of field experiments described in the literature led us to suggest that (Z)-9-tricosene may function as an aggregation pheromone bringing males and females together and as a female sex pheromone inducing mating behaviour in males.

In Chapter 7 we describe studies on the oviposition behaviour of female houseflies. We found that the females preferred to deposit their eggs in clusters at one and the same site in crevices on rough substrates even when no additional visual or chemical stimuli were present. The reason for this may be that eggs laid in crevices and clusters are less susceptible of desiccation and predation. The smell of decaying organic material attracted gravid females and induced oviposition, probably because these substrates may be suitable food sources for the larvae. In addition, we obtained strong indication that the odour of eggs collected within 30 min after oviposition was attractive to gravid females and stimulated oviposition suggesting the presence of an oviposition stimulating pheromone. However, eggs which were 2-6 h old, did not enhance oviposition, which suggested that this pheromone may disappear shortly after oviposition because it is very volatile or may disintegrate.

As far as we are aware this was the first indication of the presence of an oviposition pheromone in the housefly. The application of an oviposition pheromone at suitable locations in the flies' habitat may offer new possibilities for environmentally friendly control of houseflies. It is evident that control of the flies at moments within their life cycle when new generations are created may attack the problem -nuisance of the flies- in the most effective way. Removal of individuals from the population at a moment they do not cause annoyance and are not yet able to propagate is a more efficient way of control than removal of adult individuals. Further studies are necessary to identify the pheromone.