Site of sex pheromone biosynthesis in the female housefly, *Musca domestica* L.[1]

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Summary. The primary site of sex pheromone biosynthesis in the female housefly is the abdominal integument tissue, whereas the contribution of the legs is relatively minor. A substantial portion of the sex pheromone components are, however, transferred from the abdomen to the legs, probably as a result of grooming.

The presence of a sex pheromone on the surface of the female housefly *Musca domestica* was first reported by Rogoff and coworkers[3]. Components of the pheromone are (Z)-9-tricosene[4], a series of methyl branched alkanes[5] which potentiate the activity of (Z)-9-tricosene[6,7], 9,10-epoxytricosane, and, (Z)-14-tricosene-10-one[8]. A number of studies have substantiated the role of (Z)-9-tricosene as a major component in the sex pheromone[9–12].

Work with excised tissue and radiolabeled precursors suggested that the most likely site of pheromone biosynthesis was abdominal integument tissue[13]. In contrast, Schlein et al.[14] recently reported that the site of production and release of the housefly sex pheromone is a unicellular gland located on the legs of the female. Because of the discrepancy in these 2 reports, studies on the site of pheromone biosynthesis were undertaken and the results are reported herein.

Groups of 5-day-old virgin females (Fales strain T-II) were sacrificed by freezing, various body parts excised, and the surface lipids, which include the pheromone components, extracted and analyzed by GLC[5]. (Z)-9-Tricosene was quantitated by comparison to an internal standard of *n*-docosane. The results showed that approximately 4 µg/insect (Z)-9-tricosene was present on 5-day-old females of this strain and distributed as follows: abdomen 1.04 ± 0.04 µg (mean ± SD, n = 4), thorax 0.8 ± 0.35 µg, wings 0.60 ± 0.10 µg, head 0.33 ± 0.10 µg, and legs 1.23 ± 0.11 µg. The distribution of the other pheromone components was similar to that of (Z)-9-tricosene.

It has been demonstrated that [1-^{14}C]acetate efficiently labels all of the sex pheromone components and that [1-^{14}C]propionate was incorporated almost exclusively into the methyl branched hydrocarbons[13]. Therefore, studies using these precursors were undertaken to determine the site of pheromone synthesis. Virgin females were injected with 1 µl of [1-^{14}C]acetate (1 µCi/µl) or 1 µl of [1-^{14}C]propionate (1 µCi/µl) into the abdomen followed by a 2-h incubation at 30 °C. A 2nd group consisted of virgin females whose legs were removed prior to injection of the labeled substrates. A 3rd group consisted of intact virgin females which were injected with labeled precursors, incubated for 2 h, and before extraction, the legs were removed and bodies and legs extracted separately. Isolation and analysis of labeled products was performed as described earlier[13]. The results show that insects with their legs removed still synthesize hydrocarbon (80% of control insects) and non-hydrocarbon components (90% of control insects) (table 1). The distribution of label from [1-^{14}C]acetate between the saturated and unsaturated fractions was almost identical in both intact and legless flies, and analysis of the alkene fraction by radio-GLC[13] showed that the alkenes produced by both intact and legless insects were identical (fig. A, B). (Z)-9-Tricosene was the major alkene produced in both cases. In addition, the non hydrocarbon components produced were the same in both experiments and consisted of the C_{23} epoxide and ketone (fig. C, D). These results clearly indicate that although a significant portion of the pheromone components are present on the legs of the female housefly, the legs are not the primary site of pheromone biosynthesis.

The most likely explanation for the relatively large amount of pheromone on the legs is grooming activities of the female. To examine this possibility, 1 group of insects was immobilized with high vaccuum silicone grease and legs were extended from the body and immobilized. A 2nd group of females was immobilized in the same manner except that the legs on the right side were allowed to be free. Each group was then either injected with [1-^{14}C] acetate as described above or [9,10-^{3}H]-(Z)-9-tricosene was applied to the surface of the abdomen in hexane (1 µl). 2-h incubations were used, and then right and left legs and bodies were extracted separately and analyzed. Less than ¼ of the total pheromone synthesized was recovered on the legs (table 1). When legs were free and allowed to contact the body, more pheromone was recovered on the legs. This strongly suggests that grooming activities transfer much of

Table 1. Incorporation of [1-^{14}C]-acetate and [1-^{14}C]-propionate into the cuticular lipid-pheromone components of the female housefly[a]

Group	[1-^{14}C]-Acetate Hydrocarbon (cpm)	% Saturated hydrocarbon	NHC	[1-^{14}C]-Propionate Hydrocarbon	% Saturated
Intact insect	7546 ± 914[b]	63.5 ± 3.5	875 ± 100	1614 ± 189	98.6 ± 0.1
Legs removed	5785 ± 698	60.3 ± 1.5	780 ± 61	1159 ± 171	98.8 ± 0.3
Intact insect					
Legs	9236 ± 915	54.7 ± 2.5	1448 ± 227	839 ± 232	–
Body	12189 ± 1430	55.0 ± 2.7	2173 ± 400	1074 ± 212	–
Immobilized insects					
Body	9312 ± 2290	60.3 ± 0.6	1456 ± 234	–	–
Left legs, immobilized	1773 ± 305	59.0 ± 1.7	276 ± 26	–	–
Right legs, immobilized	1489 ± 147	62.3 ± 2.1	262 ± 17	–	–
Body	8973 ± 1819	62.7 ± 1.5	1483 ± 223	–	–
Left legs, immobilized	1345 ± 283	59.0 ± 2.6	275 ± 32	–	–
Right legs, free	6099 ± 1776	60.0 ± 2.8	800 ± 133	–	–

[a] 1 µCi of the labelled substrate was injected into the abdomen of female insects and after 2 h, the material extracted and analyzed as described in the text. [b] Mean ± SD, n = 3.

the pheromone to the legs. These results were confirmed by experiments using [9,10-^{3}H]-(Z)-9-tricosene layered on the abdomens of the insects. Only in insects whose legs were free to contact the abdomen was there any appreciable transfer of the (Z)-9-tricosene to the legs (table 2).

In vitro studies provided further evidence that the major site of pheromone synthesis was the abdominal integument tissue. Excised tissue was incubated with either [1-^{14}C]acetate or [1-^{14}C]propionate in a phosphate buffer. Following a 2-h incubation, lipids were extracted by the method of Bligh and Dyer[16] and the hydrocarbons isolated as described above. The results of these in vitro studies clearly show that the hydrocarbon components of the sex pheromone are predominantly synthesized by abdominal integument tissue (108±49 pmoles acetate/2 h/insect incorporated into hydrocarbon). Less than 6 pmoles acetate/2 h/insect was incorporated into hydrocarbon by the legs, head, thorax or fat body. Similar data were obtained using [1-^{14}C] propionate. Radio-GLC confirmed that the (Z)-9-tricosene was a major hydrocarbon component produced by abdominal integument tissue. The very small amounts of hydrocarbon produced by excised leg tissue may be due to an inability of the substrate to reach the biosynthetically active tissue in the legs, or conversely, that much of the pheromone recovered in the legs of immobilized insects in the in vivo studies was transferred there via the hemolymph.

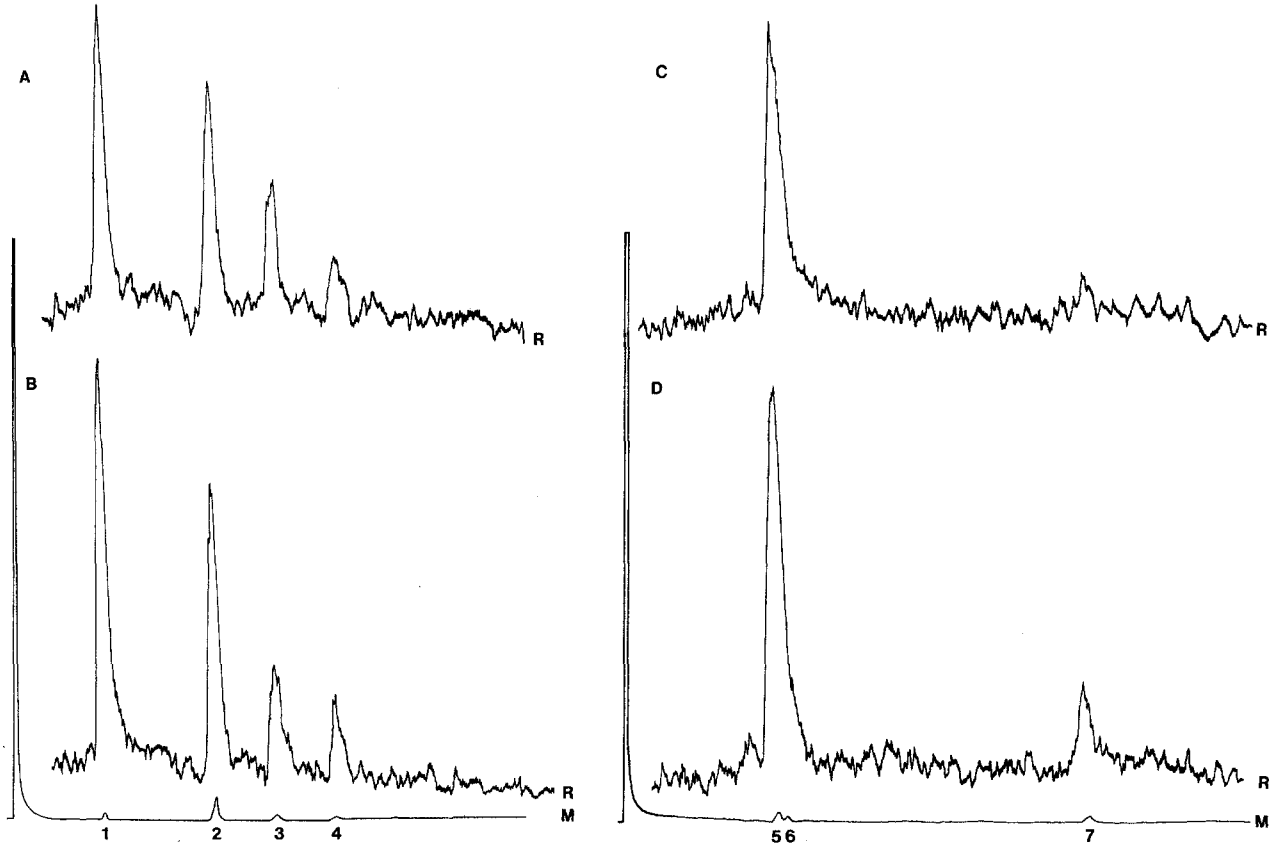
Schlein and coworkers[14] based their conclusion that the legs produced the housefly pheromone entirely on bioassay data. We have now directly measured the biosynthesis of the well characterized pheromone components using radiotracer techniques and the results show that the major site of sex pheromone biosynthesis is the abdominal integument The possibility that an as yet unidentified pheromone component may be produced by unicellular glands on the legs may explain the apparent discrepancy between this report and that of Schlein et al.[14].

The sex pheromones of a number of musciid and tsetse fly species are non-polar components[4,6,8,17–21] that are structu-

Table 2. Distribution of label from [9,10-^{3}H] (Z)-9-tricosene in the female housefly[a]

	% of label recovered	Distribution of label (%) Body	Legs
Free insects (zero time)	87.6±2.3	99.7±0.2	0.3±0.2
Free insects (2-h incubation)	80.8±5.8	90.6±0.3	9.4±0.3
Immobilized insects (all legs immobilized)	79.4±3.1	98.7±0.4	1.3±0.4
Immobilized insects (1 set of legs free and the other set immobilized)	74.7±3.9	93.6±3.5	Free 5.7±3.7 Immobilized 0.7±0.7

[a] The labeled substrate (0.2 μCi/insect) was applied to the abdomen in hexane. The legs were removed and the body and legs extracted separately and radioactivity assayed. [b] Mean±SD, n=3.



Radio-GLC of the alkenes and non-hydrocarbon cuticular (NHC) lipids of the female housefly after the incorporation of [1-^{14}C]acetate. Radio-GLC was performed as described elsewhere[13]. *A* Alkenes synthesized by intact insects, *B* alkenes synthesized by legless insects, *C* NHC lipids synthesized by whole insects, *D* NHC lipids synthesized by legless insects. The components in chromatograms A and B are identified as (Z)-9-tricosene (1), (Z)-9-heptacosene (2), (Z)-9-nonacosene (3), and (Z)-9-hentriacontene (4), and the components in chromatograms C and D as 9,10-epoxytricosane (5), (Z)-14-tricosen-10-one (6), and a C_{38} wax ester (7). M is the mass trace and R is the radioactivity trace. (Z)-9-Tricosene, 9,10-epoxytricosane, and (Z)-14-tricosen-10-one are pheromone components.

rally similar to the cuticular lipids[22,23]. Cuticular hydrocarbons are synthesized by tissue associated with the cuticle[13,24-26]. Therefore, it is likely that the biosynthesis of the hydrocarbon sex pheromone components by abdominal epidermal tissue may be common in Dipteran species.

1 Acknowledgment. This work was supported by the Science and Education Administration of the U.S. Department of Agriculture under grant No.7801064 from the Competitive Research Grants Office. Contribution of the Nevada Agricultural Experiment Station, Journal Series No 524. Housefly pupae were kindly supplied by Dr Ted Shapas, S.C. Johnson and Son, Inc., Racine, Wisconsin.

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Sex recognition pheromone in the tsetse fly *Glossina pallidipes* Austen[1]

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8 April 1981

Summary. Sexual responses of adult male *G. pallidipes* towards baited decoys show that a contact sex pheromone for this species is present in the hydrocarbon fraction of the adult female cuticle. Results are consistent with the view that the pheromone is a C_{35} compound and is present in sufficient quantity in newly emerged females to elicit maximum responses from males. Thus, maturation of sexual responsiveness is considered to be behavioural in females of this species.

Glossina pallidipes Austen is an important vector of trypanosomiasis and in the dry savannah regions of central southern Africa shares its habitat with, and will feed on the same hosts as, *G. morsitans morsitans* Westwood[3]. Both *G. m. morsitans* and *G. pallidipes* are attracted to stationary models in proportions which enable the sex and species present in an area to be estimated[3]. The numbers of flies attracted visually to such models are greatly enhanced by the provision of an olfactory stimulus such as ox odour[3] or a mixture of CO_2 and acetone[4].

It is generally accepted that locally high concentrations of tsetse around host animals provide the situation for mating to occur[5], while the attraction of non-hungry *G. pallidipes* females to hosts and a variety of baits resembling hosts has been interpreted as indicating a mate-seeking response by females of this species[3,6]. Nevertheless, the presence of specific chemicals in the cuticles of female *G. m. morsitans* which elicit copulatory behaviour by males on contact[7] suggests that correct species identification is achieved by the male. In *G. m. morsitans* the contact sex pheromone or aphrodisiac is a single hydrocarbon component which has been identified, characterized and synthesized[8]. However, it is recognised that the chemical signal will only be effective when presented to the male on an object which possesses certain physical attributes of a female fly, namely its shape and size[9]. Evidence has also been obtained that no 2 species of *Glossina* are likely to share a sex pheromone structure in common[10], and this is important for species which share the same environment and hosts.

In the laboratory, *G. pallidipes* do not mate as readily as *G. m. morsitans* and only recently have techniques been developed to overcome this problem[6,11]. Female *G. pallidipes* are most receptive at the time of ovulation of the 1st mature oocyte, when they are 9 days old at a maintenance

Table 1. Sexual responses, scored subjectively as 0, 1, 2, 3 of ⩾ 9-day-old male *G. pallidipes* to decoys consisting of killed male *G. morsitans* and *G. pallidipes* or killed female *G. pallidipes* of different ages

Decoy Species	Sex	Age (days)	N	Test male responses (No.) 0	1	2	3
G. m. morsitans	♂	7	24	24			
G. pallidipes	♂	7-8	10	10			
G. pallidipes	♀	0	21	1	1	1	18
G. pallidipes	♀	2-4	15			4	11
G. pallidipes	♀	8-10	35				35
G. pallidipes	♀	52	10			1	9

N = number of males tested. Between 3 and 5 males used per decoy in individual tests.