

were used for electron spin resonance (ESR) studies using an RZ 1306 radio-spectrometer. The methods followed were the same as those described earlier^{2,3}. Chlorophyll was measured following Arnone⁴.

Results. The results are shown in the table. While in seedlings maintained in continuous light (treatment 5, figure, a) the apices were clearly differentiated into inflorescences, in those kept in continuous darkness (treatment 1, figure, b) or those which received 6 days in darkness followed by 2 in light (treatment 2) or 2 days' light followed by 6 days' darkness (treatment 8) the apices were still vegetative, with transitional stages in other treatments as is evident from the length of the apex (table). The relative

Development of the apex, relative values of PS-I and PS-II, and greening of cotyledons in buckwheat seedlings exposed to different light and dark treatments

Treatment*	Length of apex (μm)	Relative values per mg fresh wt of cotyledons		Total chlorophyll mg/mg fresh wt of cotyledons
		PS-I	PS-II	
1. (8D+0L)	142 ± 2	0	1.38	0.003
2. (6D+2L)	142 ± 4	7.17	3.33	0.091
3. (4D+4L)	271 ± 8	15.76	9.46	0.102
4. (2D+6L)	282 ± 16	21.94	13.10	0.199
5. (0D+8L)	542 ± 2	24.60	8.04	0.225
6. (6L+2D)	214 ± 8	21.53	14.46	0.154
7. (4L+4D)	200 ± 6	18.31	11.49	0.096
8. (2L+6D)	142 ± 2	7.57	5.08	0.084

*Figures in parentheses indicate days in dark D and in continuous light L and the sequence of light treatments following seed germination.

amount of PS-I in the cotyledons also increased with increasing days of light exposure. Photosystem-I was completely absent in seedlings kept continuously in the dark (treatment 1). The amount of total chlorophyll also showed similar trends.

The dependence of floral induction on a primary chemical event during photosynthesis has been reported by many workers⁵, and a higher energy demand of apices during the onset of the generative phase is also well established⁶. The results reported here confirm our earlier presumption², that probably the onset of the generative phase involves energy absorption and a transfer of excitation to reaction centres, and subsequently primary chemical events during photosynthesis. The electron transfer during energy absorption probably serves as a prelude to further molecular events leading to the onset of the generative phase. Identification of these early biophysical events may be easy only in plants with a very short juvenile phase like buckwheat.

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The legs of *Musca domestica* and *Glossina morsitans* females as the site of sex pheromone release¹

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Summary. The site of sex pheromone release in *Musca domestica* and *Glossina morsitans* is the legs. Unicellular glands restricted to the tarsi and to the tibia are proposed as the source of the pheromone. The structure of the glands is described.

Sex recognition pheromones which initiate mating behaviour in the male fly at short range or in contact with females or baited decoys have been described for several muscid and tsetse fly species. In all investigated species, the pheromones were associated with the nonpolar cuticular lipid extracts of adult female flies²⁻⁸. We present evidence pointing at the site of pheromone excretion as restricted to unicellular glands located on the flies' legs.

Experiments comparing attraction of male flies to whole female flies and to decoys treated with extracts of female body parts are described. The experiments with houseflies followed the general lines of 'pseudofly' decoy technique of Rogoff et al.⁹. *Musca* males and females were separated on the 1st day after emergence and kept at 24–26 °C, 60–80% relative humidity.

In order to demonstrate that the site of pheromone excretion is the legs, it was important to prevent contact between legs and wings and body. For this reason, female flies were immobilized immediately after eclosion by fixing them to a small piece of cardboard. Legs were straightened and placed on a drop of melted paraffin, and wings were similarly attached to each other. The restrained females

were capillary fed on 10% sucrose solution for at least 2 days. Males used in mating tests were older than 3 days. Fly extract was prepared by placing 20 females or parts of females in 1 cm³ absolute ethanol (Merck A.R.) for about 20 h. Extract equivalent to 2 female doses was placed on shoelace knot decoys. After drying, the decoys were placed in 9-cm plastic petri dishes lined with filter paper, containing 5 males. Mating behaviour (number of strikes) was observed during 1 h.

Glossina morsitans pupae were kept at 28 °C and 70% relative humidity. The procedure and scoring method in the tsetse experiments, adopted from Carlson et al.¹⁰ took into account the fact that tsetse flies are not very active. Sexes were separated at eclosion. Males older than 7 days were placed in individual glass tubes of 2.5 × 7 cm. A test object, decoy or female fly, was introduced into the tube. The tube was tapped and moved and several contacts between male fly and the object were observed. Decoys were pieces of cork 2 × 3 × 10 mm with female extract (1 cm³ hexane, Merck A.R./10 flies). The mating behaviour of the male fly with decoys or with female flies of different ages was recorded: No response was scored as 'nil'; a short arrest of

the male's movement on the decoy was scored as 'one'; attempt at copulation, the maximal response, was scored as 'three'.

The reaction of housefly males to decoys treated with different female extracts proved that the attractant of immobilized females was restricted to the legs. Males were attracted both to decoys treated with extracts of legs or extracts of whole immobilized females, while decoys treated with extracts of bodies of immobilized females without legs had no stimulatory effects (table 1). In contrast, decoys treated with extracts of bodies without legs in females where normal leg contact with the body had previously taken place were very attractive, as were decoys treated with leg extracts of these females.

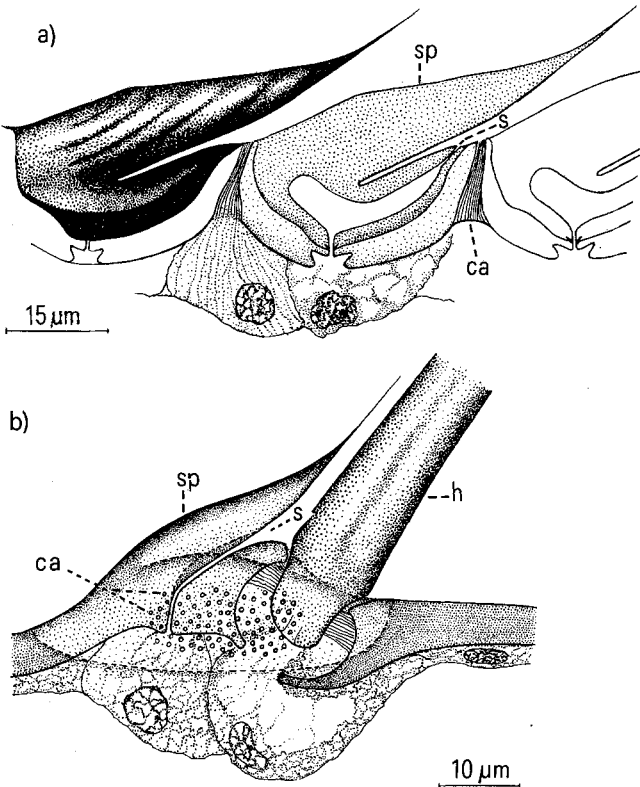
Musca domestica females elicit mating behavior by 2 days⁹. However, we found *G. morsitans* females to be attractive even prior to eclosion; puparia were opened, and pharate-adult females, several h before eclosion, were tested. At this stage, flies could move their legs but the moulting fluid was not yet completely absorbed. Pharate-adult females elicited male responses in more than 95% of the trials, differing from responses to eclosed females only in intensity (table 2). Male response to legless pharate adult females was only circa 15%. In females with legs removed 2 h following eclosion, the male response level was 90%. Legless pharate-adult females were not attractive even when live flies were left to dry for several h and the moulting fluid was absorbed. Hence the difference in attractiveness between legless pharate-adult females and the 2-h ones is attributed to the eclosed flies having moved about and rubbed their legs, thus presumably having spread pheromone over the body surface.

There is an indication that the quantity of the pheromone is very small at eclosion and increases during the first days;

extract equivalent to 12 newly eclosed flies was less potent than extract of 2-day-old females (table 2). Presumably, the pheromone on the living fly was more specifically concentrated than on the decoy, and therefore elicited a stronger response. The results of the tsetse fly experiments, though not as straight-forward as those with houseflies, support the notion that in this fly also the site of pheromone excretion is in legs of the female.

The legs of both *M. domestica* and *G. morsitans* have a large number of gland cells whose opening on the body surface is at the base of special setae or spines. Tsetse fly spines were previously described^{11,12} without reference to function. They are predominantly found in regular dense rows in which the bases almost touch each other. The rows are concentrated on tarsi and tibia of all 3 legs, but some were found on the proximal part of the femur. The number of spines was much greater in females than in males, circa 1350:420, respectively. Each spine has an outer tapering spike-like part which is continuous with, and bent over, a wide and long base, embedded in the cuticle of the leg (figure, a). The base of the spine is hollow and its cavity is connected by a short tube to a gland cell lying in a concavity at the base. There is a slit opening to the exterior at the anterior part of the base of the spine. In flies immediately following eclosion, the narrow slit between the outer spike and the base of the spine contained translucent material. Between the bases of these spines there are groups of cuticular canaliculi which are outlets for secretions of another type of gland cell.

In the housefly, the rows of setae are less regular than in tsetse flies, and the numbers of setae in males and females were not very different. There is more variability in the structure of the spines, and spines differ in morphology from those of the tsetse: in *Musca*, the secretory structure comprises a seta (on the tibia) or spine (on the tarsus) and a spike which is close and parallel to the shafts of the above. The spike narrows from a broad base, as in the thorn of a rose. The slit, the outlet of a glandular cell, opens in the



Secretory structures on the mid-tibia of female flies. a *Glossina morsitans*; b *Musca domestica*. (ca, canaliculi; h, hair; s, slit; sp, spike).

Table 1. The response of *Musca domestica* males to different female extracts on shoe lace decoys*

Extract	No. of males	Average no. of strikes per male
Untreated flies without legs	40	13.5
The legs of untreated flies	30	10.5333
Legless flies immobilized at eclosion	60	0.2666
Legs of flies immobilized at eclosion	60	13.333
Control - ethanol	30	0.3

*Decoy dose equivalent to 2 females (see text).

Table 2. Response of *G. morsitans* males to different stages of female flies

Stage	No. of tests	Male reaction (%)			
		0	1	2	3*
Intact ready to emerge females	35	5.71	-	-	94.28
Whole female several h before eclosion	38	2.63	7.89	15.78	73.68
Legless female, 2-h-old	54	9.25	-	-	90.74
Ready to emerge females without legs	111	35.13	8.10	0.9	55.85
Legless females several h before eclosion	46	84.78	4.34	6.52	4.34

*For explanation see text.

Table 3. Response of *G. morsitans* males to female extract on cork decoys

Extract	Quantity of extract (female equivalents)	No. of tests	Male reactions (%)			
			0	1	2	3
Intact females more than 1 week old	2	69	7.24	2.89	23.18	66.66
Legs of ready to emerge females	12	20	10	50	40	-
Ready to emerge females without legs	12	20	70	30	-	-

cuticle between shaft and spike. Many canaliculi of other gland cells open at the lateral margins of the base of the spike (figure, b). Structures similar to those of *Musca* were also observed in *Stomoxys calcitrans*, which has also been reported to secrete a sex recognition pheromone¹³. The proximity of spike and seta (in *Musca*) directs the flow of material excreted through the slit upwards, as described here for the specialized spines in the tsetse. Preening spreads the material on the body surface.

The location of these cuticular structures on the upper part of the lower tarsi and outer parts of the tibia affords maximum tactile contacts with other flies. Although direct experimental evidence is still lacking, the distribution of the glands, combined with the evidence presented here that the legs are the source of sex pheromone in housefly and in tsetse flies makes it plausible to infer that the source of the pheromone is the gland cell at the base of the cuticular seta or spine.

In an elegant set of experiments, Lang¹⁴ demonstrated the site of contact sex pheromones in the mosquito *Culiseta inornata* as the legs, but, unlike the flies, the sex pheromone remains restricted to the legs and is not spread over the body surface of the mosquito. With our finding in *Glossina* and *Musca*, presented here, it appears that the release of contact sex pheromones from the legs may be common in Diptera.

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Stretch receptors in the eye muscles of a teleost fish

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Summary. Extraocular muscles of a teleost fish, *Girella tricuspidata*, contain a predominantly phasic stretch receptor, which consists of fine beaded nerve terminals within the red portion of the muscle.

Muscle stretch receptors are well known in mammals, where they typically form part of the muscle spindle complex, and have also been described from all other gnathostomous vertebrate classes except the bony fishes. The complete absence of this type of receptor from a whole vertebrate class would be surprising, and its apparent

absence may only result from the difficulties of obtaining suitable nerve-muscle preparations in fish. A possible proprioceptive feedback of eye velocity information during induced vestibulo-ocular reflex² could indicate the presence of stretch receptors in extraocular muscles. In addition to this, the discrete straplike nature of extraocular muscles,

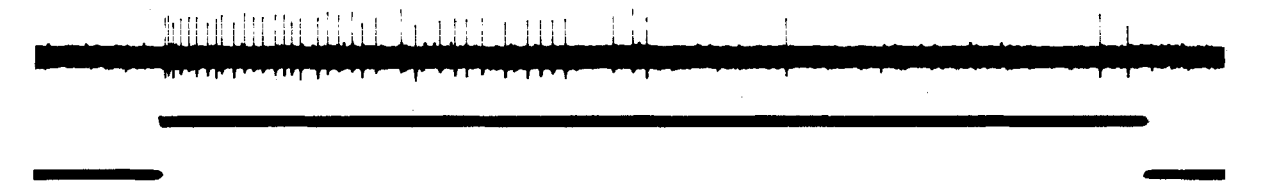


Fig. 1. Response of the receptor to an applied stretch. Upper trace: en passant electrical recording of the spike discharge; spike amplitude approximately 40 μ V. Lower trace: stimulus monitor; stimulus intensity 0.13 N, duration 3 sec.