LOCATION OF THE OLFACTORY RECEPTORS OF THE BLOWFLY *PHORMIA REGINA* MEIGEN

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ABSTRACT

The site of olfactory receptors of the blowfly *Phormia regina* Meigen and the olfactory rôle of its antennal receptors have been experimentally determined.

The capacity of nine essential oils to stimulate olfactory receptors of the blowfly has been tested and the oil of Caraway, being the best stimulant, has been employed for the present work. The response of the blowfly to vapours of this oil is not due to chemical irritation.

Olfactory receptors in *Phormia regina* are located on the antennae, labellum, and, tarsi but not on the palpi. Comparative sensitivity of the different appendages to the oil vapours has been shown. A threshold population of the olfactory receptors is necessary for the maximum degree of olfactory response.

As regards the rôle of antennal receptors, the present experiments demonstrate that, in a stationary blowfly, surface-cones are responsible for the perception of the oil-vapours.

INTRODUCTION

The location and structure of olfactory receptors of blowflies have been studied by several workers but their conclusions are not in agreement with one-another. McIndoo (1933, 1934) studied the structure and distribution of these organs in *Calliphora erythrocephala*, *Lucilia sericata* and *Phormia regina* and he concluded that the olfactory receptors are situated on the bases of the wings and legs but none on the head. On the other hand, Hartung (1935) contended that the olfactory organs of *Calliphora erythrocephala* are restricted to the antennae only. Abbot's (1938) experiments with *Cynomyia cadavarina* and *Lucilia sericata* indicated that the olfactory organs are definitely located on the head and also on other parts of the body. According to Frings (1941), however, the antennae and the labellum are the only loci of olfactory receptors in the blowfly *Cynomyia cadavarina*. In *Phormia regina* these receptors are reported to occur on the antennae, labellum and palpi (Dethier, 1952).

There is also some uncertainty regarding the identity and structure of the olfactory receptors of blowflies. Detailed structure of the olfactory pores was described by McIndoo (1934) who believed these pores as olfactory organs. But his view was disproved by later experimental work. The sensory structures of the antennae and palpi of certain species of blowflies have also been studied by various workers, particularly by Smith (1919), McIndoo (1934) and Liebermann (1926). Two types of sensory receptors have been found to occur on the antennae of blowflies : (i) *Sensilla basiconica* or surface-cones (*flächenkegel* of Liebermann, 1926) and, (ii) *Sensilla coeloconica* or pit-cones (*grübenkegel* of Liebermann, 1926). Of these, the receptors in the pits have been generally regarded as the olfactory end-organs, but no experimental evidence in support of this view has so far been adduced.

The present investigations were taken up in order to determine the site of olfactory receptors of the blowfly *Phormia regina* Meigen and, also, to demonstrate the rôle of antennal sensory structures. This work was done in the Department of Biology, John Hopkins University, Baltimore (Md.).

Materials and Methods

Freshly emerged adults of *Phormia regina* Meigen were used for the present work. The technique for measuring the olfactory response of the blowflies was essentially the same as that adopted by Abbot (1932, 1938) and Frings (1941). The blowifles were singly mounted along their backs on waxed sticks. Each blowfly was brought near the source of the odour and its proboscis-response was noted. If the odour was acceptable and attractive, the fly would respond by extending its proboscis in an attempt to feed on the stimulant (Abbot, 1932; Frings, 1941).

The first step in the present work was to select a suitable olfactory stimulant which would cause the blowflies to extend the proboscis. Fermented casein, malt extract, putrid meat and putrid eggs have been found to attract free moving blowflies in an olfactometer (McIndoo, 1934); but these substances failed to evoke proboscis response of the mounted blowflies used in the present work. Abbot (1938) tested the stimulating effect of various essential oils on the proboscis-response of mounted blowflies, *Lucilia sericata* and *Cynomyia cadavarina*. He stated that tansy oil was the best attractant. Frings (1941) trained the mounted blowflies (*Cynomyia cadavarina*) to extend the proboscis in response to stimulation by the vapours of coumarin. Dethier (1952) recorded the repellant effect of pentanol on *Phormia regina* in an olfactometer. In the present work it was considered desirable to take the proboscis-response of stationary blowflies as the criterion of olfactory perception and for this purpose it was necessary to select a suitable stimulant.

Since there is no record of the capacity of essential oils to stimulate chemoreceptors of *Phormia regina*, the following oils were tested: Oils of Caraway, Carvacrol, Celery seed, Parsley, Fennel, Rue, Dill seed, Phellandrene, and Coriander. These were obtained from Dodge and Olcott Company, New York. Each of the oils was placed in a glass vial and mounted blowflies were held, one by one, over the open mouth of the glass vial at a distance of two inches from the oil for thirty seconds and the proboscis-response was noted. For each oil fifty individuals were tested and the observations are recorded in Table I. Oil of Caraway was found to be the best stimulant. It may be noted that vapours of ethyl acetate, ethanol

Table I

Proboscis-response of Phormia regina *to essential oils*

Name of oil	Percentage of flies responding	Name of oil	Percentage of flies responding
Caraway	96	Fennel	80
Carvacrol	75	Rue	40
Celery seed	40	Dill seed	80
Parsley	36	Phellandrene	50
Coriander	16		

and a few other organic solvents also stimulated the blowflies to extend their proboscis. But this response was not sustained and evidently due to chemical irritation since it was obtained even after the removal of all the appendages like the antennae, palpi, labellum and tarsi. On the other hand, oil of Caraway failed to evoke proboscis-response from the blowflies which were deprived of all these appendages. Also, on allowing the extended proboscis to come in contact with the oil the latter was ingested, though the blowfly died soon after. These observa

tions indicate that oil of Caraway served as an olfactory stimulant and not as a chemical irritant. This oil was therefore employed in all the subsequent experiments.

In order to determine the site of olfactory receptors care was taken to maintain the blowflies in the same physiological state throughout the course of work. Freshly emerged blowflies were first given an opportunity to feed on 0.1 M sucrose solution for about 12 hours and, then, they were mounted on waxed sticks under CO_2 anaesthesia. After the blowflies recovered they were allowed to drink water to satiety so that they showed no response to water vapour. The proboscis-response of each of the blowflies to oil of Caraway was tested. *Only the individuals showing positive response were selected for further experiments* and they will be referred to as 'normal' blowflies. The flies which did not give any response were discarded.

The normal blowflies were divided into nine groups of ten each. Individuals in different groups were deprived of their appendages singly or in different combinations, as indicated in Table II, and their proboscis-response to the oil-vapour was again tested. Prior to removal of appendages the blowflies were fed on sucrose solution for two hours and, after the operation, they were allowed 12–20 hours to recover from the shock. They were allowed to drink water to satiety just before their response to the oil was determined. A period of 12 hours was found to be quite sufficient for recovery of the flies from the shock of operations. This series of experiments was repeated with five different batches of blowflies, all the individuals of a batch being the progeny of a single pair of parents drawn from a standard culture. The observations are recorded in Table II.

In order to ascertain the olfactory function of the antennal receptors the blowflies were deprived of all the appendages except the antennae and their response to the oil vapour was tested. Only the blowflies showing positive response were selected for the present experiment while others were discarded. These blowflies were divided into four groups. In one group both the antennae of each blowfly were completely coated with a layer of India Ink (waterproof) by means of a very fine brush. The blowflies of the second group were deprived of both their antennae. In the third group the pit-bearing areas on the undersurfaces of the antennae were similarly coated with India Ink. In the fourth group the surface-cones were sealed by smearing the outer surfaces and the terminal parts of the undersurfaces of the antennae with the ink. A small number of the surface-cones, situated in between the pits, was, however, left exposed. After allowing the blowflies to recover from the shock they were fed on water to satiety and then tested for their response to the oil vapours as before. The olfactory perception was measured in terms of percentage of the blowflies giving positive response.

Distribution of Olfactory Receptors on the Body

The olfactory responses of the blowflies deprived of their appendages in different combinations are indicated in Table II. Since only those normal blowflies which gave initial positive response were employed for the present work, the effect of the removal of any one or more appendages on the olfactory response of the flies would be quite definite.

The table shows that the olfactory response of the blowflies is completely abolished when all the four sets of appendages—antennae, palpi, labellum and tarsi—are removed (group IX). This suggests that centres for perception of the oil vapours are present on one or more of the amputated appendages.

In the blowflies which are deprived of the palpi but which retain antennae, labellum and tarsi (group I), the olfactory response is not in the least affected. On the other hand when all the appendages excepting the palpi are removed (group VII) the response of the blowflies is almost completely abolished. These

observations clearly indicate that no receptors on the palpi are involved in the perception of the vapours of oil of Caraway.

TABLE II

Olfactory-response of blowflies deprived of their appendages in different combinations

Group No.	Receptor areas remaining	Receptor areas removed	Experiment No.	No. of blowflies tested	No. of blowflies responding	Average percentage of blowflies responding
I	antennae labellum tarsi	palpi	i	10	10	100
			ii	10	10	
			iii	10	10	
			iv	10	10	
			v	10	10	
II	palpi labellum tarsi	antennae	i	10	9	84
			ii	10	9	
			iii	10	8	
			iv	10	8	
			v	10	8	
III	antennae palpi tarsi	labellum	i	10	7	70
			ii	10	6	
			iii	10	7	
			iv	10	7	
			v	10	8	
IV	antennae palpi labellum	tarsi	i	10	10	96
			ii	10	10	
			iii	10	9	
			iv	10	9	
			v	10	10	
V	antennae	palpi labellum tarsi	i	10	7	66
			ii	10	7	
			iii	10	6	
			iv	10	6	
			v	10	7	
VI	labellum	antennae palpi tarsi	i	10	7	70
			ii	10	8	
			iii	10	7	
			iv	10	6	
			v	10	7	
VII	palpi	antennae labellum tarsi	i	10	0	4
			ii	10	0	
			iii	10	0	
			iv	10	2	
			v	10	0	
VIII	tarsi	antennae labellum palpi	i	10	5	48
			ii	10	3	
			iii	10	4	
			iv	10	6	
			v	10	6	
IX	nil	antennae labellum palpi tarsi	i	10	0	0
			ii	10	0	
			iii	10	0	
			iv	10	0	
			v	10	0	

On removal of both the antennae (group II) or all the tarsi (group IV) there is a slight fall in the olfactory response of the blowflies. However, when only the antennae (group V) or the tarsi (group VIII) are retained and the remaining appendages removed, there is a sharp fall in the olfactory response of the blowflies ; but, still the perception of the oil vapours is quite marked (66 per cent for the blowflies with antennae and 48 per cent for those with tarsi only). This shows that receptors for the perception of oil of Caraway are located on the antennae and tarsi ; but the antennae are more sensitive than the tarsi.

In the case of the blowflies which are deprived of the labellum alone (group III) there is a marked fall in the olfactory response from 100 per cent to 70 per cent. When labellum alone is retained and all the other appendages are removed (group VI) the response is almost of the same order as that with the labellum removed but antennae and tarsi retained. These observations indicate that labellum also bears the olfactory receptors.

Olfactory Receptors of the Antennae

The sensory structures of the antennae of the blowflies *Lucilia caesar*, *Calliphora erythrocephala*, *Calliphora vomitaria*, *Cynomyia mortuora* etc., have been studied in detail by Liebermann (1926), Smith (1919) and McIndoo (1934). Two types of receptors have been recognised : (i) *Sensilla coeloconica* (Berlese, 1909) or pit-cones (*Grübenkegel* of Liebermann, 1926) which are the sensory hairs present within simple or complex pits on the antennae ; and, (ii) *Sensilla basiconica* (Berlese,

Table III

Olfactory response of blowflies with different parts of the antennae sealed with India Ink

Group No.	Receptor areas remaining	Receptor areas sealed	Experiment No.	No. of blowflies tested	No. of blowflies responding	Average percentage of blowflies responding
I	nil	entire antennae sealed	i	10	0	4
			ii	10	0	
			iii	10	1	
			iv	10	1	
			v	10	0	
II	nil	antennae removed	i	10	0	2
			ii	10	1	
			iii	10	0	
			iv	10	0	
			v	10	0	
III	surface-cones	pit-cones	i	10	8	78
			ii	10	7	
			iii	10	10	
			iv	10	9	
			v	10	5	
IV	pit-cones	surface-cones	i	10	1	20
			ii	10	0	
			iii	10	5	
			iv	10	4	
			v	10	0	

Note : Only those blowflies were used in this series of experiments which, on removal of their labella, palpi and tarsi, gave positive proboscis-response to the vapours of oil of Caraway.

1909) or surface-cones (*Flächenkegel* of Liebermann) which are the sensory hairs present on the surfaces of the antennae. The number, arrangement and size of the surface-cones and the pit-cones differ in different species of the Muscid flies. In *Phormia regina*, also, the same two types of sensory structures are found on the antennae. The pits are present on the proximal two-thirds part of the undersurface of each antenna along its lateral edges. The surface cones are present over the entire outer surface and on the terminal part of the undersurface of the antenna ; some are also present on the area inbetween the pits.

In order to determine the rôle of these structures in the perception of odour, one or the other or both types of the receptors were sealed and the olfactory response of the treated blowflies was measured as described before. The observations are given in Table III. It is clear that the response of the blowflies with completely sealed antennae (group I) and of those with the antennae removed (group II) is almost completely abolished. This shows that the antennal receptors can be effectively sealed with India Ink so that they cannot be stimulated by the oil vapours.

When the pit-bearing areas of the antennae are sealed (group III) and greater proportion of the surface-cones are exposed, olfactory response of the blowflies, though suffering a slight fall, is still very high (78 per cent) ; the slight fall in the response may be due to the sealing of some of the surface-cones present in the pit-bearing areas. This experiment shows that the surface-cones are definitely the centres for registering odorous stimuli. On the other hand, when the pits are kept exposed and the surface-cones are mostly sealed (group IV) olfactory response of the blowflies falls sharply from 100 per cent to 20 per cent, which suggests that the antennal pits are not able to perceive vapours of the essential oil. Slight response which is obtained with flies of this group may be due to the few exposed surface-cones present inbetween the pits.

Discussion

The observations recorded in the present paper demonstrate that receptors for the perception of vapours of the oil of Caraway are located on the antennae, labellum and tarsi of the blowfly *Phormia regina*, but not on the palpi.

Whether the response of the blowflies to the oil vapours is due to the stimulation of their olfactory receptors or due to irritation of the common chemical sense, needs consideration. According to Valentine (1931) and Marshall (1935) strong vapours of certain chemicals, particularly essential oils, do not stimulate olfactory receptors but cause irritation to insects through their action on the common chemical sensory cells present all over the body. Valentine demonstrated that the beetles (*Tenebrio molitor*), in which all possible centres of olfactory or gustatory receptors had been removed or sealed, still reacted to the essential oils, suggesting that such a response cannot be due to olfaction but to the stimulation of common chemical sense. Two important facts which emerge from the present work on *Phormia regina* rule out the possibility that vapours of oil of Caraway irritate the blowflies through their action on the common chemical sense : (i) Response of the blowflies is completely abolished when antennae, labellum and tarsi are removed, but remaining portions of the legs and the palpi, which are known to bear sensory hairs, are retained. If the common chemical sense was involved the amputated blowflies should have continued to respond to the oil vapours. (ii) The fact that the blowflies, when their extended proboscis is allowed to come in contact with the oil, actually ingest the stimulant, goes against the view that the flies are being irritated. They do not make any attempt to ingest ethyl acetate or ethanol, vapours of which evoke proboscis-response from the blowflies and this response persists even after removal of the appendages. In strong concentrations the oil of Caraway may serve as a repellent but not as a chemical irritant.

It is therefore certain that some specialised receptors are concerned with the perception of the oil of Caraway. Since these receptors are stimulated from a distance, it is quite reasonable to regard them as olfactory receptors.

The observation that the palpi of *Phormia regina* are not sensitive to the oil vapours is in conflict with Dethier's (1952) observations that the palpi of this species are able to perceive the repellent vapours of pentanol. Chief difference in the present work and that of Dethier lies in the nature of the stimulant employed. The discrepancy in Dethier's results and those of the present study seems to suggest that different types of chemical stimuli may stimulate different sets of chemoreceptors and receptors on the palpi may be sensitive to pentanol and other similar repellents but not to the essential oils. This point needs further investigation.

The fact that the antennae and labellum, and not the palpi, bear olfactory receptors in *Phormia regina* agrees with Frings' conclusions on *Cynomyia cadavarina*. But there is a difference in that Frings did not find the tarsi to be sensitive to coumarin vapours. This difference may again be due to differences in the chemical stimuli or in the species. However, it may be remarked that gustatory receptors have also been regarded to perceive strong vapours of certain chemicals from a distance (McIndoo, 1934; Marshall, 1935). It is not yet certain whether the sensory structures of the tarsi of *Phormia* responsible for the perception of the oil vapours are identical with or distinct from the gustatory chemoreceptors.

Another point which merits attention is the comparative sensitivity of different appendages to the vapours of oil of Caraway. It has been observed that the olfactory response of the blowflies is only slightly affected when their tarsi are removed. This might imply that these appendages do not contribute towards registering the olfactory stimuli. But when only the tarsi are retained appreciable response (48 per cent) is again obtained, showing the presence of the receptors on these organs. Similarly when antennae alone are removed there is a fall in the response of the blowflies from 100 per cent to 84 per cent while the removal of labellum alone results in a fall to 70 per cent. This apparently suggests that labellum is more sensitive than the antennae. But olfactory response of the blowflies which retain only the antennae (66 percent) is almost of the same order as that of the blowflies retaining the labellum alone (70 per cent). These results can be explained on the basis of a presumption that a certain minimum (threshold) number of olfactory receptors is required for the maximum degree of perception. The degree of olfactory response is directly proportional to the number of receptors stimulated. When either antennae or tarsi are removed the number of receptors on the remaining appendages may be just sufficient to compensate for the loss of some receptors and that is why the response is not appreciably reduced. On the other hand, when labellum alone is removed, a sharp fall in response suggests that the receptor population is markedly decreased and receptors on the antennae and tarsi fall too short of making up the deficiency. Similarly, when both the antennae and tarsi are removed and labellum alone is retained, fall in response is again sharp since the receptor population on labellum is much too short of the threshold. Fall in response of blowflies retaining only the antennae or only the tarsi can also be explained on the same basis.

As regards the rôle of antennal receptors in olfaction, pit-cones or the *sensilla coeloconica* are believed to be the olfactory end-organs by most of the workers (Röhler, 1906; Smith, 1919; Liebermann, 1926) but there has been no experimental evidence to support this view. On the basis of comparison between the biology of the Muscid flies and the condition of their antennal receptors, Liebermann (1926) suggested that the surface-cones (*sensilla basiconica* or *flächenkegel*) are capable of perceiving odour from nearby stimuli when the fly is resting and its antennae are in repose. In this condition the pit-cones do not appear to perceive the odour unless strong wind is blowing and the stimulant is directly below the antennae. He suggested that the pit-cones perceive odour when the fly is flying

and its antennae are held erect. The present experiments demonstrate that the surface-cones definitely perceive odour. The lack of perception by the pit-cones of the mounted blowflies, even when air is blown across the open end of the oil-containing vial by a fan, does not conclusively show that these receptors are not olfactory in function. For, if Liebermann's view is correct, the pits of stationary flies would not register any olfactory stimulus. It is desirable to test the olfactory function of the pit-cones in flying blowflies.

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