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OLFACTORY STIMULI AND OVIPOSITION IN THE BLOWFLY, *PHORMIA REGINA* MEIGEN

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INTRODUCTION

Lowne (1890–92) describes the blowfly, *Calliphora erythrocephala*, as ovipositing on those parts of dead birds and mammals that are not covered by hairs or feathers, or upon raw or uncooked meat. In laboratory culture, raw liver is very successful in inducing *Phormia* to oviposit. Lowne states that the female exercises discrimination in egg deposition; the number of eggs laid is proportional to the size of the cadaver.

For convenience, oviposition behaviour in the blowfly can be divided into two phases: (*a*) attraction to the general area of the oviposition site, and (*b*) the elicitation of the act of oviposition occurring after the site has been reached. In phase A both visual and olfactory cues may be used, but such evidence as there is suggests the latter are more important. West (1951) describes work by Kuzina, who studied the relative importance of taste, sight and smell in guiding the housefly, *Musca domestica*, to suitable oviposition sites. He concluded that olfactory stimuli were by far the most important, but that taste was necessary for the deposition of eggs (phase B). Vision was of slight importance in either phase. Vision, however, plays a role in site selection by the mosquito (see Wallis, R. C. (1954) for a review of the literature; O'Gower (1957)), but contact chemical stimuli perceived through tarsal chemoreceptors seem more important.

The work described here attempts to elucidate the sensory mechanisms involved in phase B. Barton Browne (1960) studied the chemical factors influencing oviposition in *Phormia* and attempted to locate the receptors involved. He found that, in the presence of sufficient odour concentration, contact stimuli (taste) played little or no part. The most important olfactory receptors involved are those on the antennae and/or palps. Dethier (1952) showed that sensilla on the antennae and palps are concerned with orienting responses, so that the sensilla involved in the two phases of oviposition appear to have the same loci. However, in discrimination experiments Barton Browne (1960) was able to show that olfactory receptors in addition to those on the antennae, palps and labellum were involved. He attributed this discrimination to receptors on the ovipositor and gave circumstantial evidence to support this view, but he did not identify the receptors. Arab (personal communication to V. G. Dethier) suggested that hairs located among the longer, thicker hairs on the anal leaflets were the receptors, while Wolbarsht & Dethier (1958) recorded electrically from the ovipositor. They decided on electrophysiological evidence that there were single neurone receptors present, but their precise location and morphology were uncertain.

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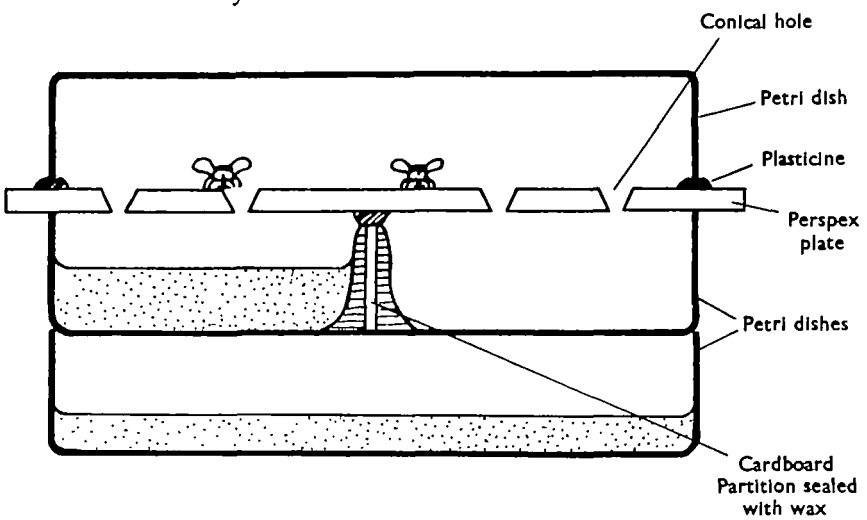
In this paper, the precise location and morphology of these receptors are described. A series of behavioural experiments to establish: (*a*) that the ovipositor receptors discovered are olfactory in function, (*b*) the relative importance of different olfactory receptors, and (*c*) the importance of tactile stimuli in oviposition were carried out.

METHODS

The blowfly, *Phormia regina* Meigen, was used throughout drawn from a laboratory culture maintained by Dr V. G. Dethier. KOH-digested preparations mounted in balsam were used to study the distribution of sensilla.



Text-fig. 1. Apparatus used in discrimination experiments. Stippling—oviposition medium. The medium in the lower Petri dish neutralizes the colour difference between the two sides of the divided dish above.

The apparatus used in discrimination experiments was modelled on that of Barton Browne (1960)—see Text-fig. 1. The flies were confined in an inverted Petri dish placed on a plastic plate. Eighteen conical holes, evenly distributed, were drilled in the plastic plate, which rested on a divided Petri dish. The halves of the Petri dish were sealed from one another so that odours could not pass from one side to the other. One side contained oviposition medium, the other was empty. A second Petri dish containing medium was placed beneath the divided one so that there was no colour difference between the two sides. As the medium below the plate was beyond the reach of the flies, it provided only an olfactory stimulus.

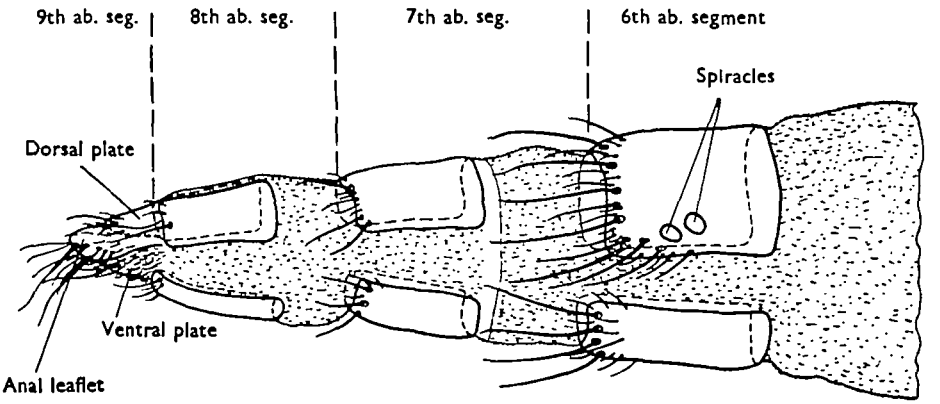
In experiments to determine the relative effectiveness of different olfactory receptors mediating oviposition, an undivided Petri dish containing medium was placed beneath the plate. No second dish to balance colour difference was then needed of course.

In experiments to determine the importance of tactile stimuli in oviposition a single, empty and undivided dish was placed beneath the plate.

For behavioural experiments newly emerged flies were cultured on sugar, liver and water in large (20 × 10 × 10 in.) gauze cages. By the 6th day the largest number of females are ready to oviposit. Females with abdomens showing signs of swelling were

selected for the experiments. Since in these experiments maximum possible egg laying is desirable, six females were placed in each container. Barton Browne (1958) has shown that social facilitation is a factor stimulating egg laying in *Lucilia cuprina*. Further, one male was included since mating may be necessary for full egg laying. Eggs are laid through the holes in the plate. The use of separate holes ensures that ovipositing females do not form groups and thus reduces interference from this complicating factor. Flies were kept in the apparatus for 48 hr. and egg laying was checked every 24 hr.

The oviposition medium consisted of 100 g. dried yeast plus 100 g. dried milk ('Klim') mixed in 1000 c.c. of tap water.



Text-fig. 2. Diagrammatic lateral view of the ovipositor of *Phormia regina* Meigen. Stippling—areas of microtrichia, plain areas—chitinized plates.

RESULTS

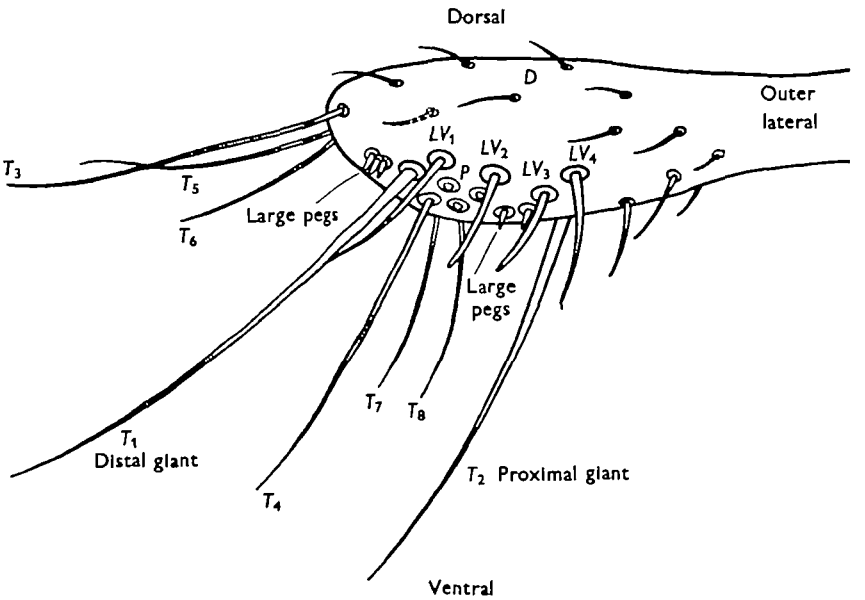
Structure of the ovipositor and distribution of sensilla

The gross structure of the ovipositor has been described by Lowne (1890–92) for *Calliphora*. *Phormia* appears to be very similar and a lateral view of the organ is shown in Text-fig. 2.

The ovipositor is normally held telescoped inside the abdomen. Morphologically it consists of the modified 6th–9th abdominal segments. Each segment bears plates of stiffened cuticle and areas of microtrichiate, pliable cuticle. Only the plates bear sensilla. All the sensilla except some of those on the anal leaflets are articulated setae (sensilla trichodea in Schenk's 1903 classification) of the type found on other parts of the body. They appear to be innervated by a single neurone terminating in the region of the socket. The anal leaflets are not plates but three-dimensional, hollow structures attached to the dorso-lateral plates of the 8th abdominal segment. The anus opens terminally between the anal leaflets, while the oviduct opens ventrally between the 8th and 9th segments.

Text-fig. 2 gives an indication of the distribution of sensilla on the ovipositor. A more detailed account is given by Wallis (1962). On the anal leaflets there are articulated setae of various sizes (Text-fig. 3), the greatest concentration being on the latero-ventral surface. The inner lateral surfaces bordering the anus are devoid of

sensilla. It is on the latero-ventral surface of the leaflets that several small pegs or cones are found (Text-fig. 3). The pegs show similarities to the sensilla basiconica of Schenk (1903) and are thin walled. There are two types: the first is short and dome-shaped with a diameter at the base of approximately $1{\cdot}5\,\mu$ and a height of $1{\cdot}5$–$2\,\mu$; the second type is larger and conical with a diameter at the base of $1{\cdot}5$–$2\,\mu$ and a height of 4–6 μ. There is a very close cluster of 3 or 4 of the large pegs distally on the leaflet—the terminal pegs. The evidence to be presented below shows that the pegs are the olfactory receptors which are involved in oviposition and from which Wolbarsht & Dethier (1958) recorded. There is no evidence at present to suggest that the smaller pegs are different in function from the larger type. The larger pegs are similar to the surface cones known to be present on the antennae and important in olfaction (Saxena, 1958).



Text-fig. 3. Distribution of hairs on the anal leaflet based on camera lucida drawings. *D*, Small dorso-lateral spines (those delineated with broken lines were found in some preparations but not others); *LV*, latero-ventral hairs, *P*, small pegs; *T* long tactile hairs.

Sensory input and oviposition

Barton Browne (1960), using apparatus similar to that shown in Text-fig. 1, demonstrated that sensilla other than those on the antennae, palps or labellum could mediate oviposition behaviour. The clear-cut distribution of egg masses on the medium side in discrimination experiments indicated to him that odour concentration beneath the plate was important in determining where the eggs were laid, since the odour difference between the two sides is much less marked above than below the plate. As the ovipositor is the only part of the fly which is inserted through the holes, he concluded that the sensilla were, in all probablity, to be found on this organ. He described localized orientation movements of the ovipositor towards a hole, presumably to an olfactory stimulus. However, he tried without success to block all olfactory input from ovipositor, labellum, palps and antennae and to demonstrate tha

ability to discriminate was lost. When the ovipositor was operated upon he found that flies did not lay eggs, though otherwise intact. His experiments do not exclude the possibility that tarsal olfactory receptors (if there be any) might function in discrimination.

The experiments described below show unequivocally that receptors on the anal leaflets are the olfactory receptors which permit discrimination even when antennal, palp and labellar receptors are blocked. A further experiment yields information on the various receptor loci which mediate oviposition.

Blockage of antennal, palp, labellar and ovipositor receptors

If, after the blockage of receptors, it can be shown that flies fail to discriminate between medium and non-medium sides of the apparatus (Text-fig. 1), then all olfactory input mediating oviposition can be presumed to be blocked.

Females anaesthetized with CO_2 and strapped down with plasticine in small dissecting dishes had their antennae and labella removed with fine irridectomy scissors. Palps were removed by crushing with jewellers' forceps. After preliminary investigation it was found that the anal leaflets could be waxed over using a low melting-point but hard wax*. This was applied with a fine, tapered glass rod heated a few mm. from the tip by a heater coil run on 6 V. a.c. through a variable transformer. The temperature at the tip could be altered by adjusting the output voltage of the latter. The ovipositor was extruded by gentle pressure on the abdomen when necessary, although it was often found that the heat from the applicator was sufficient to induce small extrusion and retraction movements of the ovipositor. The reason for this is not certain although it might indicate that warmth facilitates oviposition and is perceived locally. Surgical operations on the ovipositor are difficult to perform because of the small dimensions and local avoidance movements made by the ovipositor. Flies rarely laid eggs after surgical operation, but did so with reasonable frequency after waxing. Six females and one male (see Method) were placed together in the apparatus and left in an air-conditioned room at 70° F. (21° C.) for 48 hr. in continuous light. It was found that higher temperatures tended to inhibit egg laying. The experimental flies were provided with a little sugar solution as this stimulates activity and egg laying. Results from twelve replicates and fifteen control replicates are shown in Table 1.

It is clear that the controls show an overwhelming preference for the medium side of the apparatus. The difference is significant at the 0·001 level ('*t*' test for paired data). The number of egg batches laid was recorded following the method of Barton Browne. Because the pattern of egg laying is disrupted in experimentals and they no longer lay eggs in batches, it was necessary to know the total number of eggs laid. As the controls were run first, number of eggs per batch was not recorded for them but the average number of eggs per batch has been estimated from other identically-treated controls where the mean was 86 ($n = 142$). This gives an indication of the number of eggs involved in the control group.

The experimentals show drastically altered egg-laying behaviour. First, they fail to discriminate between the medium and non-medium sides of the apparatus. The difference is not significant. Secondly, the habit of laying in batches is broken and eggs are laid singly or in strings. Eggs are often scattered widely above the plate as

* In some cases the whole last segment was waxed over unavoidably, but flies were still able to lay eggs.

well as beneath it. The controls, on the other hand, lay virtually all eggs underneath the plate on to the medium.

Because the experimentals lay above and below the plate it is possible to analyse these results further (see Table 2). When this is done the interesting fact emerges that

Table 1. *Discrimination in flies with all olfactory receptors intact (controls) and in flies with antennal, palp, labellar and ovipositor olfactory receptors blocked (experimentals)*

(Number of batches was recorded for control flies, but the laying of eggs in batches was disrupted in experimentals and separate eggs were counted. Figures in parentheses give number of eggs estimated from other control flies. The final line gives the number of times results for the medium side are greater than those for the non-medium side, etc.)

	Controls		Experimentals	
	Medium side	Non-medium side	Medium side	Non-medium side
	Batches		Eggs	
	2	0	22	95
	2	0	15	158
	6	0	63	33
	4	0	135	64
	2	0	116	169
	3	0	106	146
	7	0	43	92
	6	0	29	50
	5	0	19	8
	2	0	117	92
	6	0	10	9
	3	0	66	87
	4	0	—	—
	3	1	—	—
	3	0	—	—
Mean	3·9 (333)	0·1 (6)	61·8	83·6
Greater	15×	0×	5×	7×

Table 2. *Distribution of eggs above and beneath the plate in the experimental group in Table 1*

	Above		Beneath	
	Medium side	Non-medium side	Medium side	Non-medium side
	22	90	0	5
	6	60	9	98
	6	13	57	20
	15	23	120	41
	71	99	45	70
	44	65	62	81
	41	66	2	26
	28	50	1	0
	15	8	4	0
	22	33	95	59
	7	5	3	4
	56	85	10	2
Mean	27·8	49·8	34	33·8
Greater	2×	10×	6×	6×

the flies actually show some discrimination above the plate but none beneath it. Results for beneath the plate where odour concentration differs most are not significantly different, but results for above the plate show a preference for the non-medium side ($P < 0{\cdot}01$). A possible explanation of this is that some visual discrimination is being shown. Normally visual discrimination, if used at all, must be very much subordinated to olfactory and perhaps gustatory stimuli. With these stimuli inoperative visual discrimination might be detectable. Although the two sides of the apparatus are supposedly colour-matched, the fact that on one side medium is viewed through the plastic plate and on the other through the plastic plate and through the bottom of the Petri dish (Text-fig. 1) results in the non-medium side appearing slightly darker and duller than the other. This factor might account for the preference for the latter above the plate. However, it is quite clear that any discrimination by olfactory means has been abolished; this means that the receptors on the anal leaflets are the ones allowing discrimination in Barton Browne's sensory deprivation experiments and not the tarsal receptors.

The importance of olfactory receptors at different sites

Since the leaflet receptors are indisputably concerned in discrimination, are they the sole means of olfactory discrimination in oviposition behaviour? They are admirably placed for this. An experiment where the anal leaflets were waxed and other olfactory receptors left intact showed that the flies discriminate perfectly. Therefore, other olfactory receptors are involved. Barton Browne (1960) has shown that these are located on antennae and/or palps and possibly on the labellum. The tarsi are shown to be unimportant above. The following experiment attempts to determine the relative importance of olfactory receptors at these different sites. The experiment is based on the assumption that the number of eggs laid will be proportional to the strength of the olfactory stimulus. Optimal olfactory stimuli will induce more egg laying than suboptimal ones. A corollary of this is that with the same olfactory stimulus progressively decreasing the number of sites at which it can act will decrease its effectiveness and decrease egg laying. That the assumption is valid is confirmed by the work of Roth & Willis (1951) which shows that the percentage response of a population of two species of *Tribolium* is closely correlated with the number of sensilla basiconica remaining on each individual after surgical operation.

Four sets of six females plus one male were set up in the simple form of the apparatus (see Method) with an undivided Petri dish containing medium. No sugar was provided since this might differentially effect egg laying in the four groups. Females showing some abdominal distension were selected; individuals selected at random from these distended females were allotted to one of the three groups of experimental flies or to the controls. If females are not selected, egg laying becomes erratic. The controls had both mid-tarsi removed as a control operation (group C), while experimentals had (*a*) antennae removed (group A), (*b*) antennae and palps removed (group P) and (*c*) antennae, palps and labellum removed (group L). Egg laying was recorded after 48 hr. in continuous light at 70° F. Results for the four groups are given in Table 3. Only each pair of results is strictly comparable, because pairs represent experiments conducted concurrently, flies being drawn from the same culture batch. The results are clear-cut and statistical analysis, considering the number of times one group lays

more than another (χ^2 test), shows that C is different from A ($P < 0{\cdot}02$), P is different from L ($P < 0{\cdot}02$), but A is not significantly different from P. Further, the 't' test for paired data shows no significant difference between A and P. Thus, egg laying is greatest in C, least in L and intermediate in A and P. The mean number of eggs per batch, however, does not alter radically. In C it is approximately 90, in A 75, in P 72 and in L 76. It can be concluded from these results that fewest sites mediating olfactory stimuli inducing oviposition are active in group L, most sites in group C. In group C all olfactory receptors are present and in group L only the ovipositor receptors are present. Since there is no difference between groups A and P, olfactory receptors on the palps (if any) play no appreciable role in oviposition. Receptors on the antennae, however, are important and their removal results in a marked drop (by half) in the number of eggs laid. Removal of palp receptors has no effect but removal of the labellum again results in a large drop in the number of eggs laid (by half).

Table 3. *The total number of eggs laid under various conditions of sensory deprivation (olfactory)*

(Normals are compared with antennaless flies, antennaless flies with flies from which antennae and palps were removed, and antennaless and palpless flies with flies from which antennae, palps and labellum were removed. Only each pair of figures is strictly comparable.)

C Controls	A Antennaless	A Antennaless	P Antennae/ palps removed	P Antennae/ palps removed	L Ant./palps/ labellum removed
1131	707	707	204	204	224
192	838	838	164	164	484
273	909	909	460	460	787
475	28	28	1	182	25
0	200	200	182	294	226
606	635	635	294	350	537
1288	245	245	350	198	0
675	180	180	198	264	0
588	0	0	264	310	0
171	0	220	310	129	0
861	220	0	129	310	0
192	0	0	310	373	173
554	0	198	373	408	0
530	198	381	408	198	136
581	0	295	198	242	377
115	381	44	242	230	191
649	295	214	230	142	0
1073	44	309	0	560	0
371	214	363	142	164	0
1056	309	141	1	60	32
111	363	12	0	851	0
299	141	12	560	307	0
350	12	—	—	—	—
596	1	—	—	—	—
284	12	—	—	—	—
Mean 520·8	237·3	269·6	228·2	290·9	145·1

Tactile receptors and oviposition

The results from the discrimination experiments indicate that tactile sensilla are present on the ovipositor. This is confirmed by electrophysiological studies. An experiment to discover whether tactile stimuli can influence egg distribution wa

carried out. It was essentially a discrimination experiment where the flies could choose to lay either above or beneath the plate in the apparatus (see Method). Tactile stimulation is provided by the holes in the plate. One unforeseen complication was that the small crack at the edge of the Petri dish cover provides some tactile stimulation also. Eggs laid in this crack were counted separately. Results are given in Table 4, which shows how the eggs were distributed between the crack and above and beneath the plate. The flies were given a little sucrose solution as food inside a ring of plasticine on the plate to facilitate egg laying and kept in constant light at 70° F. for 48 hr. and longer. Six females and one male were placed in the apparatus, the females having been selected for abdominal swelling. No medium at all was used so that no olfactory stimuli could interfere. It soon became clear that the sugar area itself could be an attractive site for oviposition. In a few cases a substantial number of eggs are laid in the sugar area, possibly obscuring the result in these instances. The second column in Table 4 shows to what extent the figure for eggs above the plate includes those in the sugar area. Only in three cases do eggs laid in the sugar area obscure the basic comparison between eggs laid above or beneath the plate.

Table 4. *Distribution of eggs in the apparatus when no olfactory stimulus is provided*

(The holes in the plate provide maximal tactile stimulation in laying eggs beneath the plate. Some tactile stimulation is provided in laying eggs in the cracks along the edges of the inverted Petri dish cover (see text).)

Above	Above minus Eggs in sugar	Beneath	In cracks
36	36	244	0
32	28	145	17
42	6	6	4
1	1	8	0
6	6	13	3
1	1	14	1
3	3	98	15
2	2	68	0
232	2	0	115
0	0	8	0
18	13	251	16
8	8	23	6
17	11	151	1
14	14	21	7
17	17	254	0
167	167	84	0
23	23	656	0
19	19	461	25
18	18	782	0
774	231	652	119
11	3	519	4
31	31	1002	4
2	2	357	1
Mean 64·1	27·9	252·9	14·6
Greater in 4 cases		Greater in 19 cases	

The table shows quite clearly that many more eggs are laid through the holes and beneath the plate than above it (columns 1 and 3). A χ^2 test (comparing number of times column 1 results are greater than column 3 results) shows the difference is

significant ($P < 0.01$); four times as many eggs are laid beneath the plate. If eggs laid in the sugar are discounted there are only two occasions when more eggs are laid above the plate than beneath it (see column 2). In fifteen cases some eggs are laid in the cracks (see column 4). The low numbers usually found there indicate that this does not provide so adequate a tactile stimulus as the holes in the plate. It is clear from this experiment that tactile stimuli can play an important role in egg distribution and also, possibly, in stimulating oviposition. However, without the olfactory stimuli it is noticeable that the number of eggs laid is often small. In eight out of twenty-three cases less than 100 eggs are laid and there are a great many cases, not included in the table, where no eggs are laid at all.

DISCUSSION

Different authors have been in disagreement over the loci of olfactory receptors on the blowfly. Thus, McIndoo (1933) concluded that they were situated on the bases of the wings and legs but not on the head in *Calliphora erythrocephala*, *Lucilia sericata* and *Phormia regina;* Hartung (1935) on the antennae only in *Calliphora erythrocephala;* Frings (1941) on the antennae and labellum of the blowfly *Cynomyia cadavarina;* Dethier (1952) on the antennae and palps in *Phormia regina* and Saxena (1958) on the antennae, labellum and tarsi of the same fly. Of possible significance is the fact that Frings used a feeding response conditioned to an odour, Dethier used a repellent and observed its effect on orienting responses, Saxena used an essential oil which induced the feeding response instinctively, while Barton Browne observed the oviposition response. Conceivably attractants and repellents might work at different loci.

The results presented here show that sensilla on the antennae, labellum and ovipositor perceive the olfactory stimuli which are important in inducing oviposition. The objection may be raised to these experiments that progressively severe operations might result in decreased egg laying. In fact, mortality is very low until after 48 hr. and there is very little difference between groups. Nearly all flies survive for 48 hr. Further, the operation performed on controls is itself fairly severe but controls show the highest egg laying. Again, there is no difference between groups A and P, although P is subjected to the severer operation. Objection to the results on these grounds can fairly confidently be discounted.

It does not necessarily follow that these antennal, labellar and ovipositor receptors are the only olfactory sensilla possessed by the fly, since other sensilla may mediate other behaviour. The results of other workers suggest, in fact, that the ovipositor sensilla mediate oviposition specifically, since they are not recorded as being involved in any other type of behaviour. On the other hand the finding that olfactory receptors are present on the antennae is in agreement with most workers, while the finding that they are present on the labellum agrees with the results of Frings and Saxena. The latter finding conflicts with that of Dethier, whose results indicated receptors on the palps but not on the labellum. However, it is possible as he himself suggests that the labellum may bear high-threshold receptors whose presence did not emerge from his data because of the type of stimulus used. With regard to the palps Hodgson (1953), working on the amphibious beetle *Laccophilus maculosus*, found that the threshold of palp receptors was much higher than that of antennal receptors. The former did not respond to air-borne vapours at the concentrations he was able to use, but responded

to chemicals in the aqueous phase. The response used as the criterion may be important here. Neither Frings nor Saxena found olfactory receptors on the palps and it is interesting in this connexion that the palps bear only pit-cones and not surface-cones. Saxena found it was the surface-cones on the antennae which were almost entirely responsible for olfaction in his experiments on stationary blowflies. The pit-cones could not be shown to perceive odour, although he thought that they might function when the animal was flying. The fact that the stimulus in Dethier's experiment was a repellent might mean that palp receptors mediate rejection principally (a negative response). The finding that olfactory receptors are present on the tarsi and wings might be explained by the view that gustatory receptors may also perceive strong vapours of certain chemicals from a distance (McIndoo, 1934; Marshall, 1935). The labellum receptors also might be of this sort, although both the above results and those of Saxena indicate that they are fairly sensitive. The difference between olfactory receptors and gustatory receptors may merely be a threshold difference.

Clearly it is not possible to come to any definite conclusions about the functioning and location of olfactory receptors until more work has been done on this topic. It is certain, however, that the receptors involved in one instinctive response in the behavioural repertoire—oviposition—are located on the antennae, labellum and ovipositor.

In the experiment where the anal leaflets were waxed over, the change in the pattern of egg laying (laying singly or in strings instead of in batches) can be partly explained by the effects of waxing. This operation interferes with the tactile hairs situated on the leaflets and last segment. These slow-adapting hairs are undoubtedly important in the process of seeking cracks and crevices in which to lay eggs and in placing the eggs together in batches (Wallis, 1962). Without this tactile information this behaviour is likely to be disrupted.

Before egg laying, flies show probing activity with the extruded ovipositor. It is significant that the head and its receptors do not appear to assist in this probing activity. The tactile hairs which are deflected and thus stimulated by this activity mostly protrude outwards at an angle from the body. Electrophysiological investigation has shown that the hairs show peak sensitivities to deflection in certain directions (Wallis, 1962) and the majority of them are slow-adapting, 'position-sensitive' sensilla. Probing into a crevice generally deflects the hairs in their most sensitive directions.

Tactile information from the ovipositor hairs aids the fly in finding cracks and crevices, but it is not a strong inducement to oviposition as the last experiment shows. Gustatory stimuli can influence egg distribution also as witnessed by the cases where eggs were concentrated in the sugar area. Of interest in this respect is the observation that single eggs or pairs of eggs were often found in the small drops of regurgitated protein (flies were fed on liver) which the flies deposited on the surface of the plate. Apparently gustatory stimuli from protein can also be important in influencing egg distribution.

During probing the ovipositor pegs are brought very close to the stimulating odours emanating from the substrate. Possibly the apparent unimportance of these receptors in other types of behaviour is a consequence of their position. When the ovipositor is retracted, as is the case during other responses, the leaflets are withdrawn into the

abdomen and sheltered by it. Diffusion of odours on to the pegs would be impeded to some extent.

It has proved possible to record electrically from the pegs. Electrophysiological studies of ovipositor receptors (Wallis, 1962) show that all receptors have too high a cuticular resistance to permit recording through a saline-filled microcapillary (Hodgson, Lettvin & Roeder, 1955) except the pegs. This is due to their relatively thin cuticle and the lower resistance they offer. It suggests they are chemosensory in function. Examples of records obtained from the olfactory pegs are shown in Plate 1. On stimulating with 0·1 M-NaCl the pegs fire vigorously. Characteristically they give multi-unit records (Plate 1 C). The different units fire at different frequencies and usually at least 3 are detectable (Wallis, 1962). Firing is persistent and irregular, but slow adaptation occurs. The pegs fire strongly to solutions of NaCl, Na_2CO_3 and $(NH_4)_2CO_3$. Possibly the pegs are normally responsive to CO_2, NH_3 and HCl. Crumb & Lyon (1921) have reported that Na_2CO_3, NH_3 and CO_2 are all stimulants to oviposition in the housefly.

From this evidence, together with the fact that they are morphologically very similar to the olfactory pegs described by Snodgrass in the bee (1925), Wigglesworth in the human louse (1941) and Saxena in the blowfly (1958), it can be concluded that these pegs are the sensilla responsible for olfactory discrimination when the antennae, palps and labellum are removed.

It is important to emphasize that stimuli influencing oviposition may play two roles. First, they may assist the fly in orientating to a suitable spot for egg laying and secondly they may actually induce egg laying. It is clear that olfactory stimuli act in both ways and are the most important stimuli. Tactile stimuli act chiefly to orient the fly while gustatory stimuli may again act in both ways. The factors which are known or suspected to influence oviposition may be summarized as follows:

Properties of the environment. Light. Moderate temperature.

Properties of the site. Olfactory stimuli (some property or properties of oviposition medium, possibly CO_2). Tactile stimuli (cracks, crevices, holes) and gustatory stimuli (sugar, protein): of lesser importance. Visual stimuli (reflectance or colour): of slight importance? (The presence of water or moisture at the site was shown by Barton Browne (1960) to be unimportant.)

Biological factors. Presence of other ovipositing females. Presence of other females? Fertilization? Previous adequate protein diet.

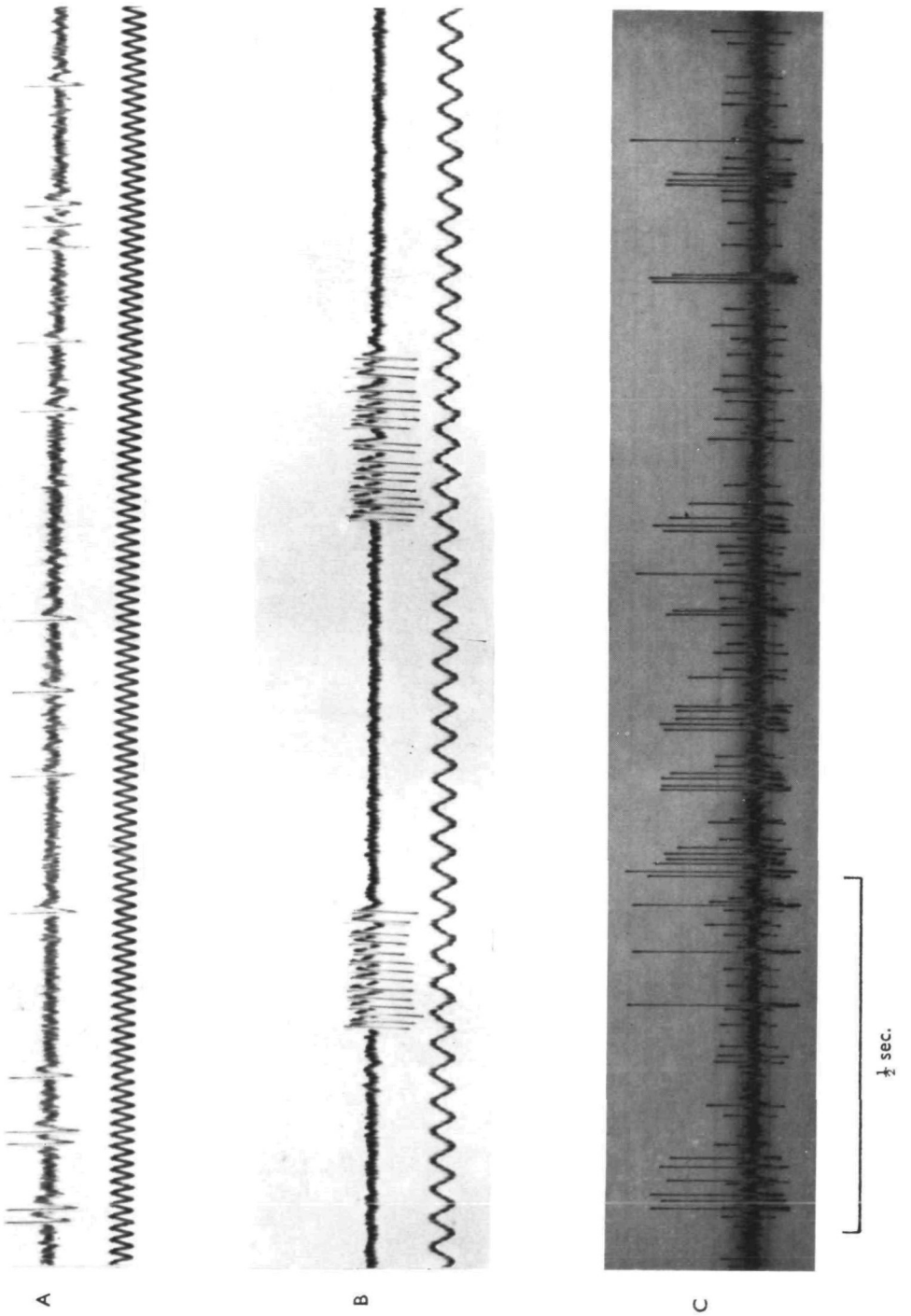
Other internal factors must, of course, be important, such as endocrine state and proprioception from the swollen abdomen.

SUMMARY

1. The work described attempts to elucidate the sensory mechanisms involved in the act of oviposition.

2. A brief account of the morphology of the ovipositor and the distribution of the various sensilla on it is given.

3. Behavioural experiments have shown unequivocally that receptors on the anal leaflets of the ovipositor are olfactory and can mediate oviposition. Flies are able to discriminate when antennal, palp and labellar receptors are blocked, but not when the ovipositor pegs are waxed over as well. A method for waxing the latter is described.



(*Facing p.* 615)

4. Sensilla on the antennae, labellum and ovipositor perceive the olfactory stimuli which are important in inducing oviposition. Possibly there are olfactory receptors at other sites which mediate other types of behaviour.

5. Tactile stimuli perceived mainly through sensilla on the ovipositor can play an important role in egg distribution and a minor role, possibly, in inducing oviposition.

6. All the evidence suggests the pegs are the olfactory receptors on the ovipositor which mediate oviposition.

7. A summary of factors known or suspected to influence oviposition is given.

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EXPLANATION OF PLATE

Electrical records from the pegs on the anal leaflets. A. A single neurone responding to 0·1 M-NaCl in a proximal peg about 1 min. after application of the stimulus. B. Bursts of responses. Terminal pegs firing to 0·1 M-NaCl. 60-cycle time-trace in A and B. C. Response of one or more of the proximal pegs to 0·1 M-NaCl soon after application. Three spike sizes can be seen.