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Segment 12.

The sensilla are on the last two-thirds, whereas the basal third lacks any type except for the whorl of fibrillae. The short bristles number about 80; they are curved and stretched out in such a way as to cover the other, more delicate sensilla. Trichoid have the maximum number of sensilla, about 100, being found close to the surface of the segment. Coeloconic sensilla are usually constant, with 8 organs in the upper half of the segment. Segment 12 also possesses one campaniform organ near the top and none of basiconic sensilla.

Segment 13.

Comparing the number and distribution of the various types of sensilla in this segment with the previous one, we find that it carries fewer small bristles, about 12, and twice as many trichoid sensilla (about 200). It bears between 24 and 32 basiconic sensilla, which are rare at the base and increase towards the apex. There are less coeloconic sensilla, between 2 and 5, with an average of three, and more campaniform sensilla with a constant number of three, two in the tips and one in the last fourth of the segment. Eight large bristles project from the colourless sub-basal area.

From the number of the different types of sensilla on the different antennal flagellar segments (Tables 1, 2 and 3), we can see that the number of each type varies in a small range in each segment.

When comparing the total number of sensilla of each type in the whole antenna of females and males we find that there are nearly twice as many trichoid, basiconic and coeloconic sensilla in the female as in the male; of campaniform sensilla there is the same constant number (6) in both sexes.

VII. Reactivity of the Normal Female Mosquitoes towards the Attracting Factors.

Before testing the behaviour of mosquitoes with certain sense organs missing towards the attracting factors, it was important at the beginning to test the behaviour of normal unoperated mosquitoes towards these factors and to ascertain the degree to which the factors differed in attractiveness.

In preliminary experiments the behaviour of the unoperated mosquitoes was tested towards an air stream carrying the following combinations of factors:

1) Low moisture content of 15-25% R.H. and temperature of 34° C.

2) Moisture content of 75-85% R.H. and temperature the same as that of the experimental room (25-26° C).

3) Body odour, associated with a low moisture content of 15-25% R.H. and a temperature of 25-26° C.

These combinations proved to have very little effect on the attraction of mosquitoes, as shown in Table 4.

TABLE 4.

Preliminary experiments on the behaviour of unoperated mosquitoes towards an air stream of 3 L/min. loaded with different combinations and degrees of the three factors [6], body odour, moisture and temperature, only one of these factors being used in each experiment, and this according to previous investigations, in its highest attractive effect.

No.	Number of experiments	Factors	Mean reactions of 40 mosquitoes	
			Alighting	Hovering
1	16	Low moisture content of 15–25 % R.H. and temperature of 34° C.	1	16
2	10	Moisture content of 75–85 % R.H. and temperature of 25–26° C.	0	8
3	10	Body odour, low moisture content of 15–25 % R.H. and temperature of 25–26° C.	0	11

The number of mosquito reactions to these combinations of factors was low and not high enough for us to be able to use such combinations on amputated mosquitoes. It is obvious that the results are more reliable when a high number of reactions is obtained in the control experiments, because there would then be a gradual decrease in reactions along with the progressive elimination of the antennal sense organs. For this reason, we abandoned those combinations in which only one factor was at its highest attractive effect and turned to using combinations with more than one factor at their highest attractive effects. Four combinations were tried:

[6] Of the attracting factors only temperature and moisture content varied in degree. Body odour was always taken from the left hand of the same test subject.

1) Body odour, accompanied by moisture content of 75-85% R.H. and temperature of 34°C.
2) Moisture content of 75-85% R.H., accompanied by temperature of 34°C.
3) Body odour, accompanied by moisture content of 75-85% R. H. and temperature of 25-26°C.
4) Body odour, accompanied by low moisture content of 15-25% R.H. and temperature of 34°C.

The result with the first three combinations showed high reactivity (see Tables 5 to 7), while the last combination gave a comparatively low reactivity with a mean number of 7 alightings and 22 hoverings in 17 experiments. This last result was still not high enough to make us use such a combination on amputated mosquitoes, and, therefore, only the first three types of combinations were adopted and used in the following experiments with amputated mosquitoes. But before we discuss the results with these combinations we should point out that in spite of all the precautions we took in our experimental technique—using mosquitoes of nearly the same age and physiological condition and carrying out all the experiments in an air-conditioned room with a constant temperature and humidity—the reactivity of mosquitoes towards the same attracting factors differed from one day to another and sometimes from one experiment to the next on the same day. On some days the mosquitoes reacted very well towards the attracting factors, while on others they reacted less and occasionally even they showed a low level of reactivity either throughout the day or just in some of the experiments carried out on a given day [7].

The reasons or factors which might account for variations in the results of the same experiments are:

1) The final record of the reactions of flying insects in a limited time of 10 minutes could change to a certain extent owing to the behaviour of the individual mosquito. As also noticed by LAARMAN (1955) a mosquito might stay on the gauze in front of the air outlet for a very long time, and so it is counted once, or else it stays for a short time, then it flies away and might return to be counted again for the second time. The same thing happens with the hovering reactions of mosquitoes. Some used to fly in front of the air outlet for a long time and others for a short time, then disappear and might appear afterwards again.

[7] No experiments were made on the days when mosquito reactivity was very low. The results of single experiments during which mosquitoes were not active enough were excluded from the final record of the day's experiments and were replaced by those of other experiments made on the same day.

2) The condition of the testing person could change from one day or from one time to another and this might affect the reactivity of mosquitoes in experiments with odour (FREYVOGEL, 1961).

3) Nevertheless, RAHM (1956) has noticed that the mosquito reactivity (*Aedes aegypti*) differs as well with the "artificial arm". This fact indicates that the mosquito reactivity might also be affected by climatic and weather factors. As we worked in a climatized room, temperature and humidity were kept constant and there would thus be no variation in the effect of these two factors on mosquito reactivity. As to the air pressure: HAUFE (1955) with his laboratory experiments on female *Aedes aegypti* found that the atmospheric pressure has a sufficiently significant effect on the flight activity of this species. The effect of decreasing pressure was more stimulating than that of increasing pressure for pressures above 735 mm, while below 735 mm the opposite relation was obtained. However, in his discussion, FREYVOGEL (1961) points out that changes in air pressure as applied by HAUFE in his experiments are unlikely to occur in nature and this author reached the conclusion that the changes in the reactivity of mosquitoes cannot be ascribed to one single weather element, whereas he definitely thinks that the mosquito reactivity is affected by changes of the weather as a whole.

It is not possible for us to say whether FREYVOGEL or HAUFE is right, since we did not ascertain the effect of atmospheric pressure alone on mosquito reactivity. What we can say, however, is that despite our climatized room (constant temperature and humidity) we still found a marked fluctuation in mosquito reactivity.

4) One must be aware that to carry on the experiments during the day, the Anopheles mosquitoes, being nocturnal insects, were placed under fairly abnormal conditions.

Owing to these and possibly other factors the results of the same experiments could change. We shall call them henceforth the "uncontrollable interfering factors".

Because of the uncontrollable interfering factors which would affect the behaviour of mosquitoes, many experiments were necessary in order to obtain a representative sample of the whole population. For this reason all the results of control experiments (carried out prior to those with amputated mosquitoes) were included together to provide an exact picture for a more or less homogeneous population. In this way we can reduce the variability due to those factors and minimise observational experimental errors.

For measuring the mosquito reactivity, the arithmetical mean obtained from 7 series of experiments (each series consisting of

8 individual experiments) was calculated. And as an appropriate measure of a mean value, we used the arithmetical mean as given by the formula

$$\bar{x} = \frac{\Sigma x}{N}.$$

This procedure was carried out in each type of experiment (or, in other words, in each type of factor combination), and the results are given in Tables 5, 6 and 7.

TABLE 5.

Reactivity of normal mosquitoes in control experiments towards an air stream of 3 L/min. loaded with body odour, moisture content of 75-85% R.H. and temperature of 34°C.

Series	Reactivity of 40 mosquitoes in 8 experiments								Total reactivity	Mean
	1	2	3	4	5	6	7	8		
1	185	190	168	155	119	157	178	188	1340	168
2	118	135	138	154	174	157	162	156	1194	149
3	126	138	139	119	151	165	144	131	1113	139
4	209	175	189	202	183	177	156	151	1442	180
5	176	151	131	137	144	125	118	120	1102	138
6	190	206	185	169	192	173	152	155	1422	178
7	132	138	167	159	128	148	138	157	1167	146

Mean reactivity ($\bar{x}$) of normal mosquitoes in 7 series of experiments = 157.

TABLE 6.

Reactivity of normal mosquitoes in control experiments towards an air stream of 3 L/min. loaded with moisture content of 75-85% R.H. and temperature of 34°C.

Series	Reactivity of 40 mosquitoes in 8 experiments								Total reactivity	Mean
	1	2	3	4	5	6	7	8		
1	72	111	89	88	58	113	87	95	713	89
2	162	151	148	125	137	113	139	135	1110	139
3	88	91	80	106	113	81	95	108	762	95
4	128	92	98	119	96	78	83	97	791	99
5	133	123	141	105	113	131	137	142	1025	128
6	89	97	119	115	101	138	140	126	925	116
7	75	115	124	112	125	132	92	108	883	110

Mean reactivity ($\bar{x}$) of normal mosquitoes in 7 series of experiments = 111.

TABLE 7.

Reactivity of normal mosquitoes in control experiments towards an air stream of 3 L/min. loaded with body odour, moisture content of 75-85% R.H. and temperature of 25-26°C.

Series	Reactivity of 40 mosquitoes in 8 experiments								Total reactivity	Mean
	1	2	3	4	5	6	7	8		
1	59	52	66	75	39	59	46	45	441	55
2	117	100	62	85	79	68	83	68	662	83
3	90	80	68	66	38	72	61	45	520	65
4	84	75	74	87	65	49	58	42	534	67
5	72	74	64	92	47	60	53	32	494	62
6	97	81	89	77	60	51	54	59	568	71
7	62	49	73	68	53	59	49	57	470	59

Mean reactivity ($\bar{x}$) of normal mosquitoes in 7 series of experiments = 66.

As a measure of dispersion, the standard deviation was calculated according to the following formula

$$s = \sqrt{\frac{\Sigma\,(x - \bar{x})^2}{N - 1}}$$ (Documenta Geigy, 1960)

where N denotes the number of series of experiments, s the standard deviation of the numbers observed, x the mean reactivity of one single series of experiments, and $\bar{x}$ the mean reactivity of 7 series of experiments.

After calculating the standard deviation for each type of experiments, the relative standard deviation (^{s} rel.) [8] expressed as a percentage of the mean reactivity ($\bar{x}$) was reduced. Like the standard deviation, the relative standard deviation measures the distribution of the invididual values around the mean; in addition, it facilitates in our case comparison of the range of variation in each type of experiments.

In order to study the effect of the different types of factor combination on attraction, the mean reactivity, standard deviation and relative standard deviation were calculated for each type. The results are given in Table 8.

From Table 8, we see that the statistical variation within each type of experiments, as shown by the relative standard deviation, lies within a range of 11.2 and 16.4%. This range of variation within each type of experiments could be attributed to the pre-

[8] This relative standard deviation is usually called coefficient of variation.

viously mentioned factors. However, this range of variation is rather narrow and at the same time it is practically common to all types of experiments; which permits comparison of the mean reactivity values.

TABLE 8.

Mean reactivity, standard deviation and relative standard deviation for the three types of attracting factor combinations.

No.	Type of attracting factor combinations	Mean reactivity ($\bar{x}$)	Standard deviation s	Relative standard deviation $s_{rel.}$
1	Body odour, moisture content of 75–85 % R.H. and temperature of 34° C.	157	17.6	11.2 %
2	Moisture content of 75–85 % R.H. and temperature of 34° C.	111	18.2	16.4 %
3	Body odour, moisture content of 75–85 % R.H. and temperature of 25–26° C.	66	9.0	13.6 %

The last table reveals a significant difference in the mean reactivity value ($\bar{x}$) between each type of experiments (as proved by the t test). In the first type, for instance, the mean reactivity value is 157 with a relative standard deviation of 11.2%, while in the second it is 111 with a relative standard deviation of 16.4% and in the third 66 with a relative standard deviation of 13.6%.

From these results we can conclude that in a constant room humidity of 50-60% R.H. and temperature of 25-26°C. the effect of human body odour combined with moisture content of 75-85% R.H. and temperature of 34°C would result in a high reactivity value (157), but when the temperature is reduced and becomes equal to the room temperature, the reactivity value decreases considerably (66). When body odour is excluded and temperature increased again to 34°C with the same moisture content of 75-85% R.H., the reactivity value increases considerably (111), although it is still much less than in the first combination. This would show that heat has a great effect on mosquito reactivity, even more than body odour (over the limited distance used in our experiments).

If we compare the two kinds of reaction—i.e. alighting, which was usually followed by probing movements, and hovering—in the different types of combination, we find, as shown in Table 9, that when the temperature was 34°C in the first and second types, the alighting reactions were more numerous than the hovering ones. The addition of body odour, as in the first combination, raised the alighting reactions from 76% to 89.3%, while in spite of the presence of body odour but with a decrease of temperature to 25-26°C, as in the third combination, the alighting reactions declined considerably to 24.5%.

In the three combinations of factors mentioned the moisture content was always constant at 75-85% R.H. But in some experiments we made (20 experiments), when the moisture content decreased to 40-50% R.H. and was combined with the temperature of 34°C, the alighting reactions were about 60% (alighting reactions 30, hovering 19). This result, compared with the result obtained from the second combination in the last table, shows that the number of alighting reactions has decreased.

From these findings we can conclude that the change of tem-

TABLE 9.

Reactions of normal mosquitoes in the control experiments towards the three types of attracting factor combinations.

No. of experimental series	Body odour, moisture content of 75–85 % R.H. and temperature of 34° C.		Moisture content of 75–85 % R.H. and temperature of 34° C.		Body odour, moisture content of 75–85 % R.H. and temperature of 25–26° C.	
	Kind of reaction		Kind of reaction		Kind of reaction	
	Alighting	Hovering	Alighting	Hovering	Alighting	Hovering
1	1222	118	532	181	85	356
2	1072	122	915	195	142	520
3	1032	81	560	202	148	372
4	1036	131	564	227	118	416
5	1338	104	786	239	132	338
6	978	124	665	260	123	371
7	1166	256	634	249	150	418
Total reactions	7844	936	4656	1553	903	2791
Total reactions in per cent	89.3	10.7	76	24	24.5	75.5

Reactions are calculated in 7 series of experiments with each type of combination, and each series is composed of 8 experiments. 40 mosquitoes were used in each experiment.

perature, in combination with body odour and humidity, has more effect on the alighting reactions than changes in the other two factors. This explains what happens in nature: at a distance odour alone is the factor reaching the mosquito and guiding it to its host, in the vicinity of the host the effect of odour is increased by the addition of body moisture. Warmth, then, is the factor responsible for the actual alighting, followed usually by the probing action.

VIII. Reactivity of Female Mosquitoes with Progressive Amputation of their Antennal Flagellar Segments towards the Attracting Factors.

The three combinations of factors which showed the high attractive effect with the normal unoperated mosquitoes were tested against groups of mosquitoes with progressive symmetrical amputation of their antennal flagellar segments. In each operation an equal number of segments from both antennae were eliminated. It was hoped that the study of the change in reaction intensity with different amputated groups of mosquitoes, would reveal the possible function of the sense organs distributed on the antennae.

In order to estimate the change in reaction caused by progressive amputation, each group of operated mosquitoes was tested with a parallel control group of unoperated mosquitoes. In each group of operated mosquitoes the number of mosquitoes involved was between 35 and 40, whereas the number of accompanying controls was fixed at 40.

When comparing the corresponding reactions we were restricted by the following facts:

1) Owing to mortality, the number of operated mosquitoes used finally in the reaction experiments was not always the same.

2) Variations due to the uncontrollable interfering factors from one experiment to another or one day to the next (see p. 34).

Hence a standard basis was necessary, in which the influence of these factors was eliminated and consequently direct comparison possible. So in each series of experiments the reactions of the operated mosquitoes were corrected to give a value corresponding to the number of control mosquitoes (40).

To exclude as far as possible the influence of the other uncontrollable interfering factors, the reactions of the operated mosquitoes were corrected with reference to a constant reactivity value (100). This procedure would allow us to compare the influence of progressive amputation on reactivity.