THE HUMIDITY BEHAVIOUR OF THE MEALWORM BEETLE, *TENEBRIO MOLITOR* L.

II. THE HUMIDITY RECEPTORS

BY D. P. PIELOU

From the Zoology Department, University of Birmingham

(With Seven Text-figures)

THE existence of sensillae sensitive to humidity has frequently been surmised, but there is no experimental evidence on their form or position in insects. Necheles (1925) suggested that the antennal hairs of the mosquito might be sensitive to humidity; Thomson (1938) made an attempt to locate the receptors in the mosquito, *Culex fatigans*, but found the experimental difficulties too great. Kennedy (1937) attempted unsuccessfully to locate the receptors in the locust, *Locusta migratoria*. Gunn & Cosway (1938) found that humidity behaviour in the cockroach, *Blatta orientalis*, became irregular when the antennae were infected with a fungus, and they suggested on these grounds that the receptors are located on the antennae. Thus there is no satisfactory evidence on the site of humidity receptors in insects. The problem has also been examined in other terrestrial arthropods. Blumenthal (1935), working on the olfactory sense of spiders, decided that certain organs on the tarsi of the legs are olfactory receptors. As he used water as one of his "odours" and found a reaction to it, he concluded that these organs were also humidity receptors. Gunn (1937) could not locate the humidity receptors in the wood-louse, *Porcellio scaber*.

In the work described here, the humidity receptors of the beetle, *Tenebrio molitor* L., have been located, and an attempt has been made to identify them. It has already been shown that this insect reacts clearly to humidity (Pielou & Gunn, 1940); the general methods used for that work proved suitable for the work described here.

LOCALIZATION OF RECEPTORS

Before the receptors can be identified, they must be located by amputation, covering with wax or similar methods. In order to facilitate such treatment, insects to be operated upon were first cooled to a temperature low enough to make them inactive; cooling was found to be more satisfactory than the use of anaesthetics. This procedure was found to have no ill effects, for after recovery unoperated animals gave the usual humidity reactions described by Pielou & Gunn (1940).

Fortunately the first organs to be examined were the antennae and maxillary palps, and the experiments at once revealed the site of the receptors.

Tests were carried out using the standard gradient (Pielou & Gunn, 1940, p. 291), in which animals are exposed to a short gradient of humidity from 94 to 100% relative humidity (% R.H.). Under these conditions, normal animals show an intense reaction, collecting on the drier side of the chamber at 94–97% R.H. and avoiding the moister side at 97–100% R.H.

The antennae of animals to be tested were amputated with scissor-forceps in such a way as to leave only the basal segment intact; that is, ten of the eleven antennal segments were removed. Eight experiments involving 40 animals were carried out on beetles treated in this way and controls with normal animals were carried out concurrently. The amputation of the antennae completely abolished the reaction to humidity and there was no recovery even after several weeks.

A similar series of experiments was carried out in which the maxillary palps instead of the antennae were amputated on both sides. Animals treated in this way gave just as intense a reaction as normal animals. It seems, therefore, that the sense organs concerned are confined to the antennae and do not occur on the maxillary palps.

It might be thought that sense organs may be present on the maxillary palps, perhaps in numbers too small to affect the intensity of reaction. This criticism is dealt with later, but it may be pointed out here that elimination of a single segment of the antenna has a marked effect on the intensity of reaction, while removal of both maxillary palps has none.

Animals without antennae behaved in a normal manner, apart from their humidity behaviour; so did those from which the palps had been removed. The insects suffered no obvious ill effects in either case.

ACTIVITY OF ANTENNA-LESS *TENEBRIO*

Experiments involving the amputation of antennae have been criticized because abnormally high or low activity may result from the operation, thus causing an apparent abolition of sensitivity to a particular stimulus, and so leading to the unjustified conclusion that the receptors concerned are located on the antennae. Several workers, including von Frisch (1921), have shown that amputation of antennae need not affect reactions mediated by receptors located elsewhere. Marshall (1935), in an extensive review of the olfactory function of the insect antenna, also came to the conclusion that amputation was a legitimate means of elimination. There is some evidence, however, that the antennae of some insects bear "stimulatory organs" (Wolsky, 1933) which affect general activity quite apart from specific stimulation. In the bug, *Rhodnius prolixus*, for example, Wigglesworth & Gillett (1934) found that if the antennae are removed the insect falls into a state of "akinesis" or inactivity from which it is not easily aroused. It was therefore necessary to examine the activity of antenna-less beetles.

The activity of normal beetles kept at various constant relative humidities is

described quantitatively by Gunn & Pielou (1940, Fig. 3). Precisely similar experiments were carried out with antenna-less animals. The differences of loco-motory activity shown by normal animals kept at different humidities were com-pletely abolished (Fig. 1), but the resultant activity level was neither very low nor
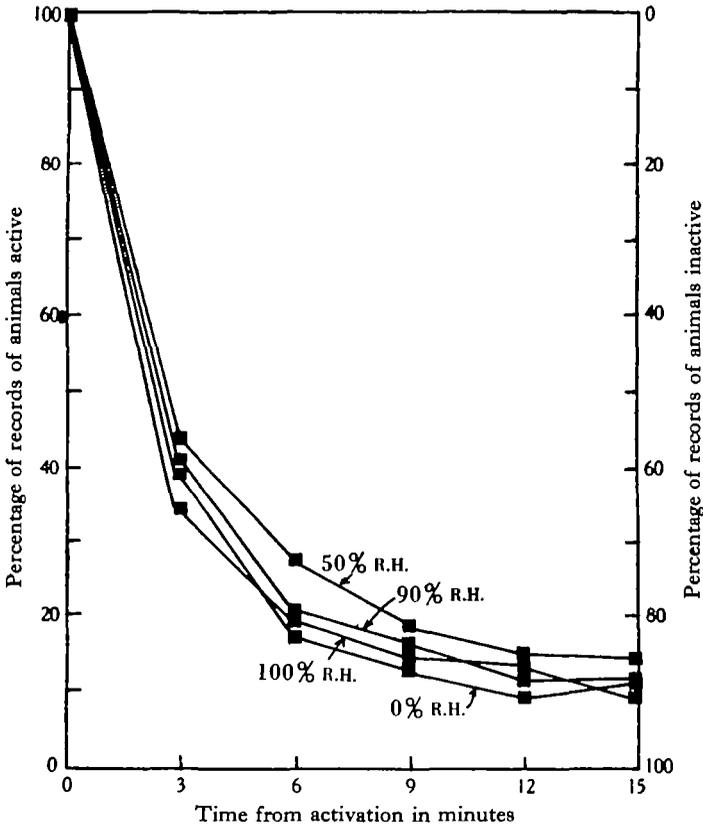

Fig. 1. Decline in activity with time in *Tenebrio molitor* deprived of their antennae and kept at various uniform humidities. The humidity does not affect activity, as it does with animals which have their antennae (see Fig. 3, Gunn & Pielou, 1940), and the activity is not depressed or increased outside the normal range by the amputation.

very high; it was below that shown by normal animals kept at 100 % R.H. and above that shown by normal animals kept at 0 % R.H.

It is therefore clear that amputation of the antennae does not have any general ill effect on the mealworm beetle and that the antennae have no considerable function as "stimulatory organs".

THE SENSE ORGANS ON THE ANTENNAE AND ON THE MAXILLARY PALPS

A morphological examination of the antennae and palps was made by Valentine (1931), who gave a description of the various sensillae; he did not give the number of each kind of sensilla. In order to get this information, whole mounts
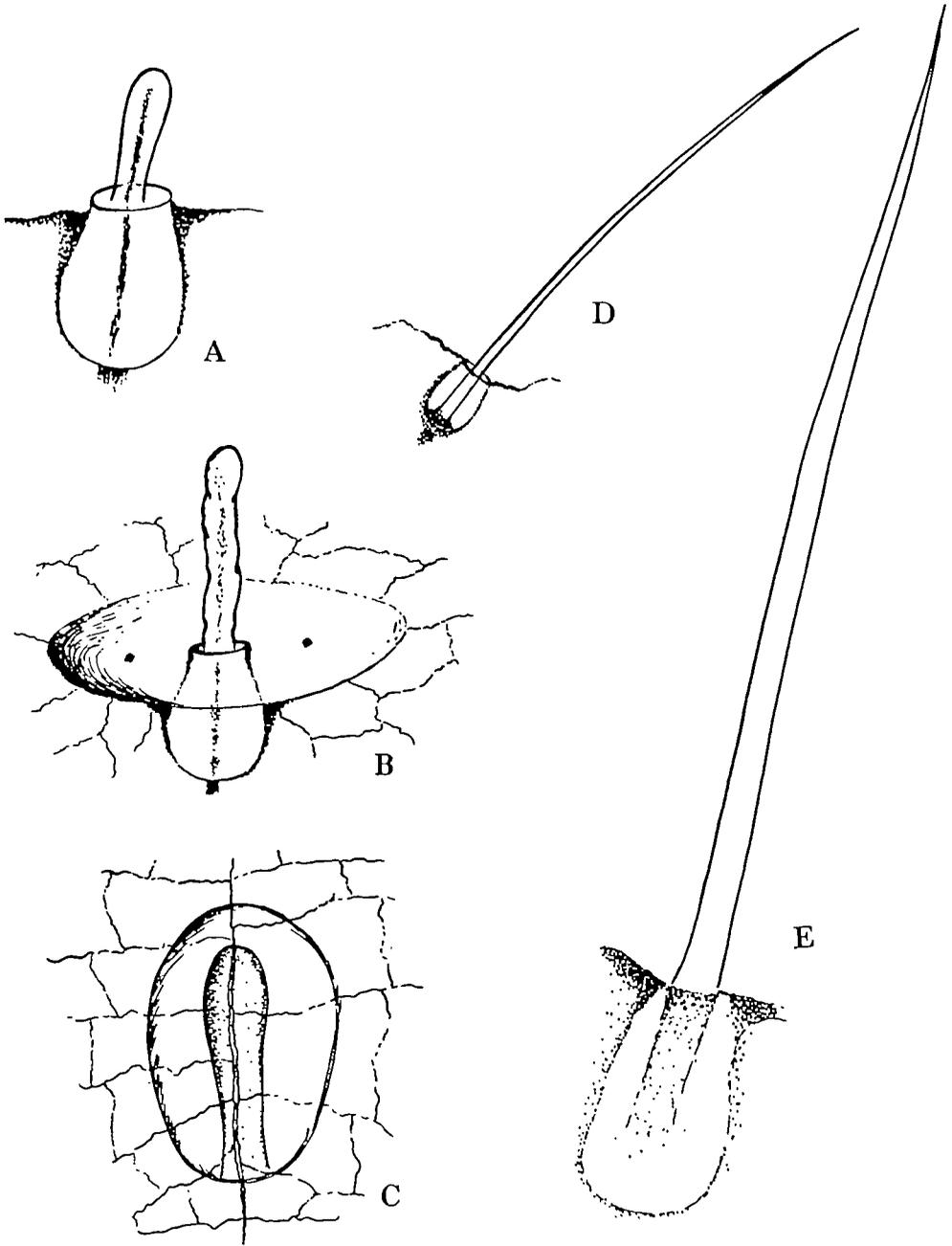
Fig. 2. The sensillae present on the antennae and maxillary palps of *Tenebrio molitor* (partly after Valentine, 1931). A, peg organ. B, pit peg organ. C, enclosed peg organ. D, hair sensilla. E, bristle. All five types occur on the antenna; only enclosed peg organs and hair sensillae occur on the palps. Hair sensillae and bristles are drawn on a smaller scale than the others.

of these appendages were made, the material being obtained from young adults which had just emerged from the pupae. The cuticle of these newly emerged adults is not yet blackened and is softer than in older animals, but all the sense organs are fully formed.

The antenna consists of eleven segments, some very slightly longer than others, but all of approximately the same form. The structures found on the antennae are described below and illustrated in Fig. 2.

(1) *Peg organs* (Basiconic sensillae). These are thin-walled pegs, the bases of which are expanded into a distinct basal bulb. They are by far the most numerous organs on the antenna and are very densely crowded on the terminal (distal) segment (Fig. 2A).
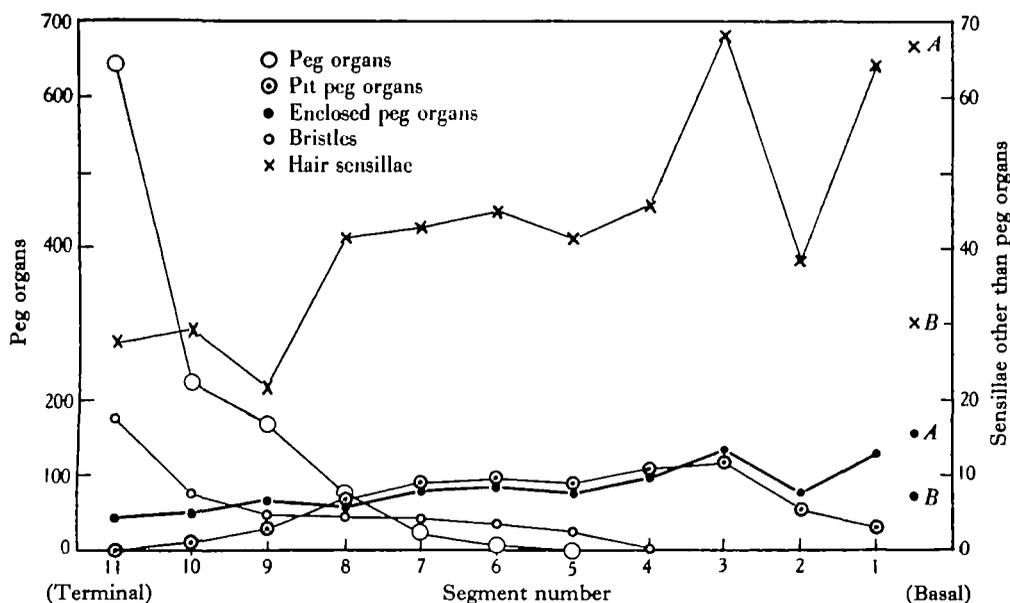

Fig. 3. Numbers of each type of sensilla on the eleven segments of the antenna of *Tenebrio molitor*. The numbers of hair sensillae and enclosed peg organs on the terminal (A) and subterminal (B) segments of the maxillary palps are also shown. N.B. Scale for peg organs on the left, scale for other sensillae on the right.

(2) *Pit peg organs* (Coeloconic sensillae). These are peg organs set in a bowl-like depression, the pit. The pegs are longer than those of ordinary peg organs. The "pit" is much wider and much shallower than in most coeloconic sensillae (Fig. 2B).

(3) *Enclosed peg organs* (Ampullaceous sensillae). Valentine (1931) describes these as peg organs set in a flask-like cavity which communicates with the exterior by means of a slit. I was able to see the slit in a few cases only; the organ generally had the appearance of being quite enclosed and cut off from the exterior (Fig. 2C).

(4) *Hair sensillae*. These are thin-walled setiform sensillae (Fig. 2D).

(5) *Bristles or spines*. These are thick-walled, long, stiff structures, much bigger than and quite distinct from the hair sensillae (Fig. 2E).

The first four of these kinds of sensillae belong to types which are usually described as chemo-receptors (Snodgrass, 1926; Eltringham, 1933; Wigglesworth, 1939). The bristles, on the other hand, are usually described as tactile, and the thickness of their walls makes it unlikely that they are chemo-receptors of any kind.

Two of these five types of sensilla—the enclosed peg organs and the hair sensillae—are found on the maxillary palps, where they are indistinguishable in form from those on the antennae. Since amputation of the palps does not affect the reaction to humidity, it is unlikely that they carry any humidity receptors, so the enclosed peg organs and the hair sensillae can be eliminated. None of the five types of organ occurs in identical form elsewhere on the body.

The quantitative distribution of these sensillae was obtained by counting them on each segment of twenty-four antennae, mounted whole. The results are shown in Fig. 3, the points representing averages from the twenty-four antennae. A much smaller scale has been used for peg organs because of their very large numbers. No significant difference was found in the numbers for the two sexes. In Fig. 3 the numbers of enclosed peg organs and hair sensillae on the terminal (A) and sub-terminal (B) segments of the maxillary palps are also shown.

It will be seen that the peg organs are confined to the distal six segments of the antenna, and the bristles to the distal seven segments, while pit peg organs occur on each segment except the terminal one and enclosed peg organs and hair sensillae are present on every segment.

INTENSITY OF REACTION WITH PROGRESSIVE AMPUTATION OF ANTENNAL SEGMENTS

Various numbers of segments of the antennae were cut off, equal numbers from the two antennae, in the manner already described. The intensity of reaction was measured in the usual way, using the standard gradient chamber (Pielou & Gunn, 1940). The results are shown in Fig. 4, where each point is the mean of 20 experiments (100 animals, 4000 position records). The line drawn on each side of each point represents twice the standard error of the mean. The measure of intensity of reaction is here the excess percentage of records for the drier side $\left(\dfrac{100\,(D-W)}{D+W}\right)$ (Pielou & Gunn, 1940).

From Fig. 4 it appears that there is a significant reaction still remaining when seven pairs of segments have been removed from the antennae, but not when eight pairs have been removed. With seven pairs of segments removed (four pairs left) the peg organs and the bristles are all gone (Fig. 5), so neither of these can be the sole humidity receptors, while with eight pairs of segments removed (three pairs left) there are still pit peg organs present; there are also hair sensillae and enclosed peg organs left, but they also occur on the maxillary palps and have already been eliminated for that reason. It was found that amputation of both palps did not diminish the intensity of reaction either with antennae intact or with the reaction already reduced by partial amputation of the antennae.

Consequently, we are driven to conclude that the pit peg organs are hygro-receptive. These sensillae occur not only on the fourth segment but also on the first three (Fig. 5), but when only three segments remain on each side there is nevertheless no reaction to humidity. This suggests that there may have to be a
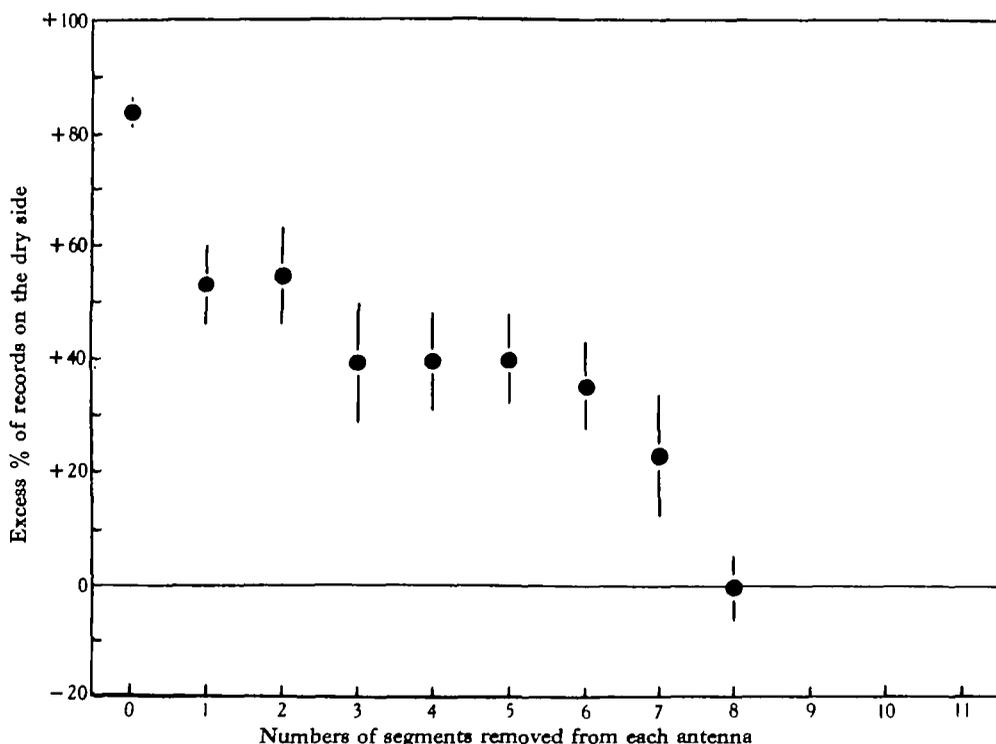

Fig. 4. Intensity of reaction (excess percentage), in the standard gradient of humidity, shown by *Tenebrio* with various numbers of antennal segments amputated symmetrically. Each point represents the mean of twenty experiments and the vertical line represents twice the standard error of the mean on either side. There is a reaction with seven pairs of segments removed but none with eight pairs removed.

certain threshold number of sensillae present before a reaction can occur, and the number on the three basal segments may be too low. This possibility was investigated by means of asymmetrical amputations (see below). Even so, pit peg organs cannot be the sole hygro-receptors, for while there are none of these sensillae on the eleventh segment, amputation of this segment reduces the intensity of reaction considerably. This question is discussed below.

ASYMMETRICAL AMPUTATION OF ANTENNAL SEGMENTS

These experiments were carried out in exactly the same manner as those involving symmetrical amputations. Amputation of the whole of one antenna, leaving the other intact, did not cause circus movements (Gunn & Pielou, 1940).
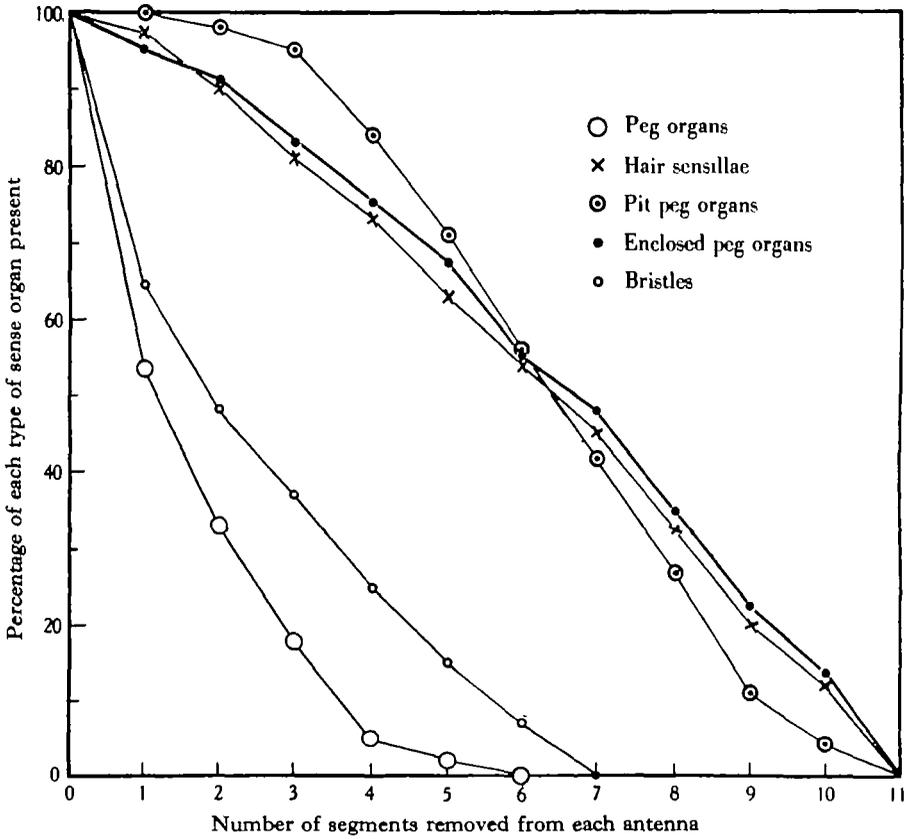
Fig. 5. Proportion of each type of sensilla remaining as the antennal segments of *Tenebrio* are progressively amputated symmetrically. There is still a reaction to humidity when seven segments have been removed, but not when eight have been cut off.
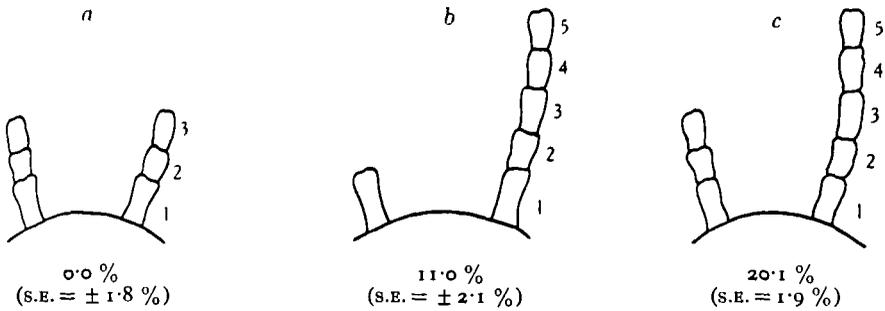

Fig. 6. Diagrams to show the numbers of antennal segments remaining in the experiments demonstrating the occurrence of humidity receptors on the basal segments, and the results of those experiments. Thus, in the experiment represented by *b*, the intensity of reaction was 11 % (S.E. ± 2 %).

The results are expressed diagrammatically (Fig. 6). Fig. 6*a* shows that with three segments remaining on each side (total of six segments remaining) there is no reaction to humidity. Fig. 6*b* shows that when six segments remain asymmetrically —five on one side and one on the other—a significant reaction is obtained. This is quite in keeping with the previous results, since we know from them that receptors are present on segments 4 and 5, but perhaps absent from segments 2 and 3. The crucial experiment is shown in Fig. 6*c*. If segments 2 and 3 bear no receptors for humidity, animals with three segments left on one side and five on the other should show the same intensity of reaction as animals with one left on one side and five on the other, as in Fig. 6*b*. In fact, however, the presence of the segments 2 and 3 does lead to a significantly more intense reaction. Humidity receptors are therefore undoubtedly present on these segments, although experiments after symmetrical amputation did not reveal them.

These experiments remove the objection to regarding the pit peg organs as hygro-receptive. Unfortunately for simplicity, these organs diminish in frequency anteriorly and there are none at all on the distal segment. The pit peg organs cannot therefore be the *sole* humidity receptors. In the distal region of the antennae, the only other possible structures are the peg organs—which are very similar to pit peg organs—and the bristles, which are thick-walled structures most probably tactile and protective in function and unlikely to be humidity receptors. As the distribution of these two types is very similar in *Tenebrio molitor*, however, it is not possible to devise crucial experiments to decide between them with certainty, but it may be possible in some other species.

Further experiments involving the asymmetrical amputation of the most distal segments made it quite certain that the presence of the terminal segment, on which there is the most dense concentration of peg organs, is very important in determining the intensity of reaction.

THE PROBABLE RECEPTOR STRUCTURES

From the lines of evidence presented above, it appears that the sensillae sensitive to humidity are the pit peg organs and the peg organs. These are both thin-walled sensillae of the type which in general appears to be chemo-receptive; in *Tenebrio molitor* Valentine (1931) identified the peg organs as olfactory receptors. The two functions are not necessarily inconsistent with one another. The slight possibility that the thick-walled bristles are hygro-receptive as well as or instead of the peg organs has not been excluded experimentally.

POSSIBLE MODE OF ACTION OF HUMIDITY RECEPTORS

A receptor sensitive to humidity may function in the same way as an olfactory receptor, the water vapour being regarded as an "odour", although this is rather begging the question, for very little is known of the mechanism of olfactory receptors. This mode of action is implied by Blumenthal (1935). It is more likely

that a humidity receptor acts like a hygrometer. For instance, the receptor may act like an atmometer, evaporation of water taking place at a rate which depends on the dryness of the air and resulting in quantitative changes of chemical concentration, osmotic pressure or mechanical stress in the receptor cells. Again, the receptor might act like a wet bulb thermometer, evaporation resulting in a lowering of temperature in special cells and being detected by heat receptors. In instruments working on any of these principles, saturation deficiency or the evaporating power
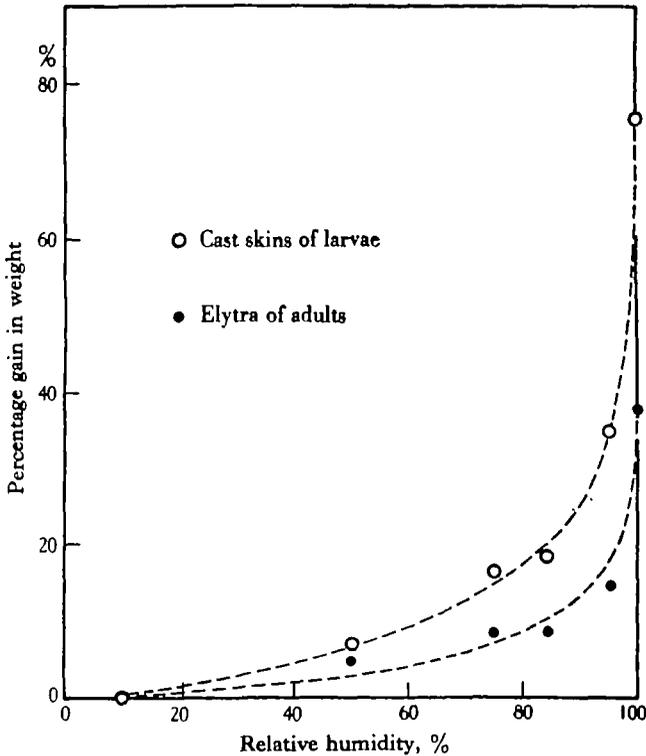

Fig. 7. Hygroscopic properties of samples of dead cuticle from *Tenebrio molitor*. The material was kept at each humidity until constant in weight. The weights are expressed as gains over the weight at 10% R.H.

of the air would be a more suitable measure of humidity than relative humidity; relative humidity could be estimated only when additional factors had been assessed.

It has been shown, however, that the reaction of *Tenebrio* is more closely correlated with relative humidity than with saturation deficiency (Pielou & Gunn, 1940). The only hygrometers recording relative humidity directly depend on the hygroscopic properties of materials like hair and paper, and Necheles (1925) and Thomson (1938) have suggested that mosquitoes have hygro-receptors working on this principle. The hygroscopic properties of dead cuticle of *Tenebrio* were therefore examined to see if this material is suitable for hygrometry. The parts chosen were

the elytra of adults and the cast skins of larvae. The material was kept at constant humidity until its weight had become constant, it was then transferred to another humidity and weighed, and so on. Fig. 7 shows the results. It will be seen that the cuticle is markedly hygroscopic, and that its weight is most sensitive to humidity changes in the upper part of the humidity scale. For a 10% change of relative humidity, the weight changes 20–40 times as much above 90% R.H. as it does below 80% R.H. These curves are strikingly similar to those relating the intensity of reaction of *Tenebrio* to the relative humidity (Pielou & Gunn, 1940, Figs. 1, 2).

The dead cuticle comes very slowly to its equilibrium weight (some hours to one day), while the thin-walled hygro-receptors (pegs) must be supposed to do so very quickly, for the behaviour reaction approaches completion in about 15 min. (Pielou & Gunn, 1940). The main difficulty arises, however, in trying to imagine how the hygrometrically effective part of the cuticle can be more affected by the humidity of the atmosphere than by the water inside the body.

SUMMARY

1. Removal of both antennae of the mealworm beetle, *Tenebrio molitor*, completely abolishes the animal's reaction to humidity. Removal of both maxillary palps has no effect on the reaction.

2. The quantitative distribution of the five types of sensillae present along the eleven segments of the antenna is described. Two of these types occur also on the maxillary palps.

3. Progressive symmetrical amputation of antennal segments leads to a gradual reduction of the humidity reaction to zero; there is a reaction with four segments remaining on each side and no reaction when only three segments remain.

4. Asymmetrical amputation of the more basal segments shows that humidity receptors are nevertheless present on these and that a threshold number of sensillae must be left in action before a reaction occurs.

5. The experimental work, taken in combination with the observations in the distribution of sensillae, shows that the pit peg organs are hygro-receptive. Either the peg organs or the bristles or both are hygro-receptive as well. It is, however, unlikely that the bristles are hygro-receptive.

6. Experimental work on the locomotory activity of this beetle shows that the conclusions are not invalidated by any general ill effects of amputation of antennae.

7. It is suggested that the hygro-receptors function hygroscopically.

REFERENCES

BLUMENTHAL, H. (1935). *Z. Morph. Ökol. Tiere*, **29**, 667–719.
ELTRINGHAM, H. (1933). *The Senses of Insects*. London.
FRISCH, K. VON (1921). *Zool. Jb.* Abt. allg. Zool. Physiol. **38**, 449–514.
GUNN, D. L. (1937). *J. exp. Biol.* **14**, 178–86.
GUNN, D. L. & COSWAY, C. A. (1938). *J. exp. Biol.* **15**, 555–63.
GUNN, D. L. & PIELOU, D. P. (1940). *J. exp. Biol.* **17**, 307–316.

KENNEDY, J. S. (1937). *J. exp. Biol.* **14**, 187–97.

MARSHALL, J. (1935). *Trans. R. ent. Soc. Lond.* **83**, 49–72.

NECHELES, H. (1925). *Arch. Schiffs.- u. Tropenhyg.* **29**, 288–91.

PIELOU, D. P. & GUNN, D. L. (1940). *J. exp. Biol.* **17**, 286–94.

SNODGRASS, R. E. (1926). *Smithson. misc. Coll.* **77**, 1–80.

THOMSON, R. C. M. (1938). *Bull. ent. Res.* **29**, 125–40.

VALENTINE, J. M. (1931). *J. exp. Zool.* **58**, 165–228.

WIGGLESWORTH, V. B. (1939). *Principles of Insect Physiology.* London.

WIGGLESWORTH, V. B. & GILLETT, J. D. (1934). *J. exp. Biol.* **11**, 120–39, 408.

WOLSKY, A. (1933). *Biol. Rev.* **8**, 370–417.