

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

## I. THE REACTION TO DIFFERENCES OF HUMIDITY

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(Received 23 February 1940)

(With Four Text-figures)

HUMIDITY is well known to be a factor of great importance in the lives of many insects and other terrestrial arthropods. Apart, however, from the pioneer work of Shelford (1913) and his school on the rather complex stimulus of "evaporating power of the air", there has been, until recently, little detailed and exact study of the behaviour of animals towards humidity as a stimulus. The recently developed methods of Gunn & Kennedy (1936) now make such study possible. By means of these methods, humidity behaviour has been examined in the wood-louse, *Porcellio scaber*, by Gunn (1937); in the locust, *Locusta migratoria*, by Kennedy (1937); in the mosquitoes, *Anopheles gambiae* and *A. funestus*, by De Meillon (1937); in the mosquito, *Culex fatigans*, by Thomson (1938) and in the cockroach, *Blatta orientalis*, by Gunn & Cosway (1938). The work described below deals with the reactions of the adult mealworm beetle, *Tenebrio molitor* L., towards humidity. This beetle lives in flour, bran and similar products and is moderately resistant to desiccation. It loses about 4% of its weight per day in dry air at 25° C. and dies in 8-9 days. Its resistance to desiccation is therefore less than that of its larva, which was investigated by Buxton (1930).

### METHODS

The apparatus used in this work was the alternative chamber described by Gunn & Kennedy (1936). In this chamber, the humidity is higher on one side than on the other, and the number of animals observed on each side is noted. The humidities are controlled by sulphuric acid-water mixtures (Wilson, 1921) and the extreme values of the humidity gradient thus set up in the chamber are measured with Edney hygrometers. The animals can be offered a variety of humidity alternatives, varying both in the difference between the upper and lower extremes in a chamber and in their specific values. Thus a difference of 20% relative humidity (R.H.) can be provided either with specific values of 90 and 70% R.H. or with specific values of 30 and 10% R.H.

<sup>1</sup> Apart from preliminary experiments, all the experimental work was done by D. P. P.

The apparatus was used in the manner described by Gunn (1937), in which five animals are used in one chamber and forty observations made at 15 min. intervals, thus yielding 200 position records in each experiment. Each experiment lasts 10 hr. and extends over 2 days. About a dozen experiments can be carried out concurrently under these conditions. After each observation the animals are activated mechanically, by pushing them with a cotton covered wire (pipe-cleaner). Each set of position records shows the number of animals on the drier and on the moister sides of the chamber; records of animals in a narrow middle zone, which cannot properly be assigned to either side, are put into a separate category.

The intensity of reaction shown is expressed in two ways: (1) as the ratio of position-records on the drier side to those on the wetter side ( $D/W$  ratio; Gunn, 1937) and (2) as the excess percentage of records on the drier side ( $\frac{100(D - W)}{D + W}$ ) (Gunn & Cosway, 1938). In both expressions, the few records for the middle zone are ignored. In the former case, the theoretical value for no reaction at all is 1.0, while in the latter case it is 0.0%. In Table I is shown a set of data from some

Table I. *Results obtained from four typical experiments at 25° C.*

Index no. of exp.      ...      ...      ...	A. 29	A. 47	B. 28	D. 3
Relative humidities in the chamber (% R.H.):				
(a) Highest	29½	48	85	99½
(b) Lowest	9½	11	42	92½
(c) Difference (a - b)	20	37	43	7
Number of records in each zone:				
Drier (D)	130	114	160	191
Moister (W)	69	76	33	9
Middle	1	10	7	0
Intensity of reaction:				
(1) As ratio $D/W$	1.9	1.5	4.9	21.2
(2) As excess percentage	30.5%	19.0%	63.5%	91.0%

typical reactions. The data for a particular series of experiments, under conditions which give an intense reaction, are shown in Fig. 3 and will be referred to later.

Usually, for each experiment, there was a control in which all four dishes in the chamber contained a single concentration of sulphuric acid, so that the humidity was uniform throughout the chamber. Any collection of animals on one side in such a control was due to chance or to factors other than humidity. The controls corresponding to the experiments of Table I are shown in Table II. In a total of 119 controls, the average mean intensity of reaction was very close to the theoretical expectation ( $D/W = 0.97$  instead of 1.0; an excess percentage of -1.5 instead of 0.0%). Twice the standard deviation of the average mean excess percentage of all controls was  $\pm 18.4\%$ , corresponding to a ratio of  $D/W$  of 1.45 or of  $W/D$  of 0.69. Thus in a single experiment, if the ratio obtained is over 1.45, the probability is at least 21 to 1 that a reaction has taken place.

In these experiments the two sexes were kept separate, having been separated in the pupal stage with the aid of the diagnostic features described by Hein (1920b).

Table II. *Results obtained in controls corresponding to the experiments shown in Table I*

Index no. of exp.      ...      ...      ...	A. 29	A. 47	B. 28	D. 3
Relative humidity in the control chamber (uniform throughout)	19½%	40%	63½%	90%
Number of records in each zone:				
"Drier" ( <i>D</i> )	96	103	89	110
"Moister" ( <i>W</i> )	99	95	97	87
Middle	5	2	14	3
"Intensity of reaction":				
(1) As ratio " <i>D/W</i> "	0.97	1.08	0.90	1.26
(2) As excess percentage	-1.5%	4.0%	-4.0%	11.5%

This separation was necessary because Valentine (1931) has demonstrated a reaction to sex smell in *Tenebrio molitor*. In order to avoid any chance of desiccation, cultures were kept as moist as possible, consistent with avoiding infestation by mites or fungi; on the day before an experiment, the animals to be used were kept in a saturated atmosphere and provided with water to drink. De Meillon (1937) and Gunn & Cosway (1938) have shown that in some insects the direction of the reaction to humidity (i.e. positive or negative) depends on whether the animals have been desiccated or not; in this work on *Tenebrio* the possibility of desiccation has been excluded. The insects were reared at 25° C., using methods given by Hein (1920a) and Buxton (1930).

The experiments were carried out in a constant temperature room at 25 ± 0.5° C. and under constant artificial light; the intensity of the light varied from 22 to 42 metre-candles at different points on the benches.

#### GENERAL RESULTS

In nearly all the experiments the majority of the animals were seen to be on the drier side, whatever the humidities available; in no case was there a significant excess on the moister side. A typical set of results has been shown in Table I. The results of the whole series of experiments are shown in Fig. 1, in which the ratio *D/W* is the measure of the intensity of reaction. The highest relative humidity available in the chamber is plotted against the intensity of reaction; the number near each point indicates the difference between the highest and the lowest humidities available in the chamber in that particular experiment. For instance, if the highest relative humidity was 30% R.H. and the number by the point is 20, then the lowest humidity in that experiment was 10% R.H. The unbroken line indicates the theoretical value for no reaction and the broken line shows the value equal to twice the standard deviation obtained from controls.

From this graph, two things are apparent. First, the intensity of reaction is very clearly determined by the specific value of the highest humidity in the chamber. Second, the extent of the range available—i.e. the difference between the highest

and lowest humidities available, or the steepness of the gradient—is not important in determining the intensity of reaction. When the upper humidity is below 70% R.H., the reaction, although significant, is always slight however great the difference of humidity available; but above 70% R.H. the reaction increases rapidly in intensity and becomes maximal when the highest humidity available

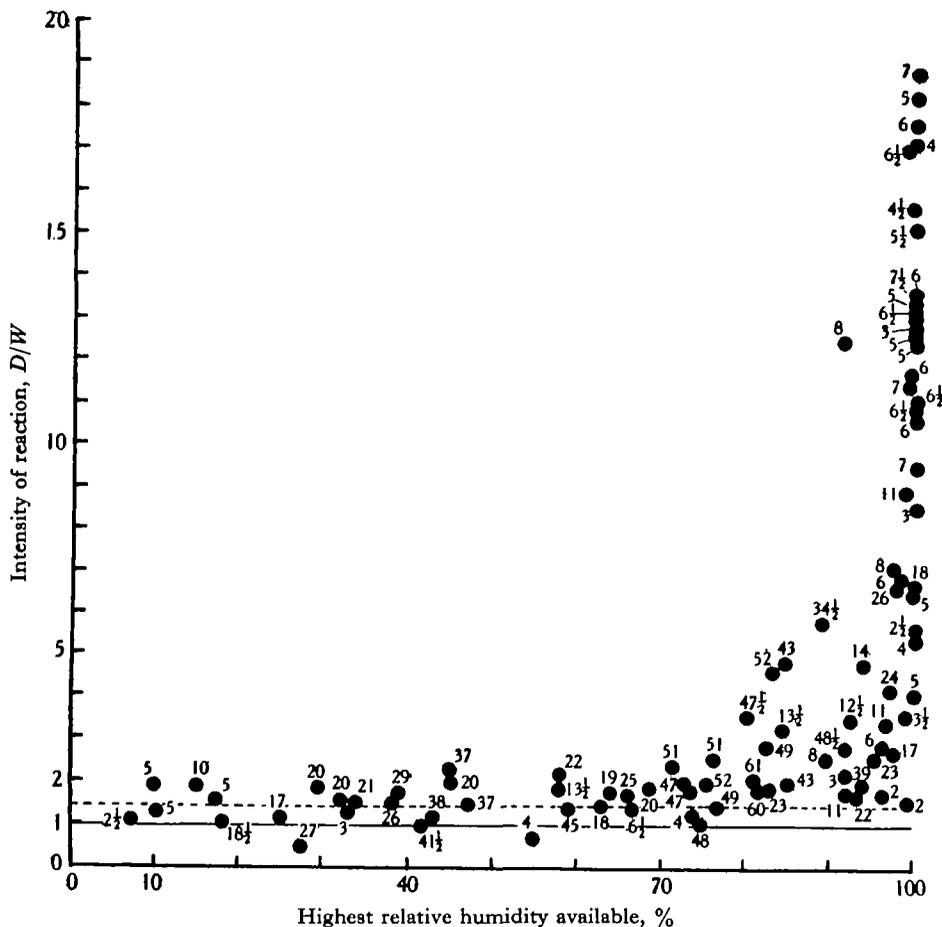
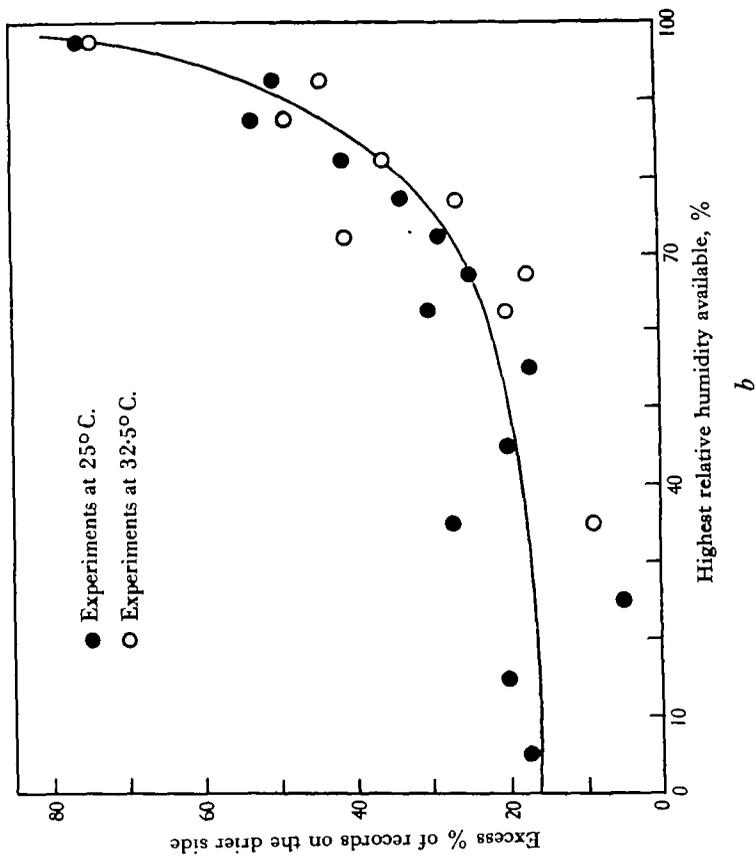
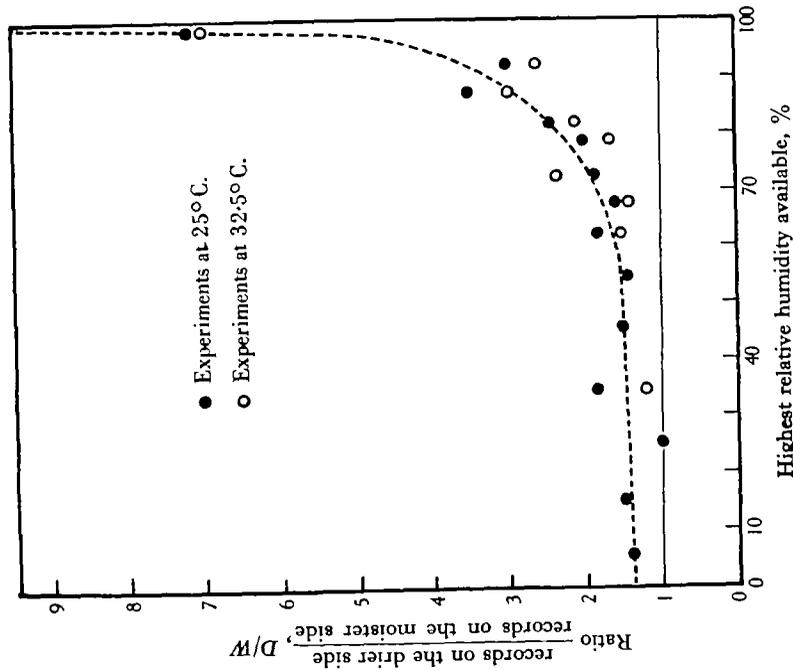


Fig. 1. Results of separate experiments, showing intensity of reaction—expressed as a ratio  $D/W$ —developed under various humidity conditions. The figure by each point gives the difference (% R.H.) between the highest and lowest humidities available in the chamber in the corresponding experiment. There is a reaction at all humidities, always towards the dry side, but it becomes intense only when the highest humidity available is very high.

is 100% R.H. In other words, the higher the value of the highest humidity, the more intensely it is avoided. Since the extent of the range of humidity available in a chamber is of little importance in determining the intensity of reaction, the data of Fig. 1 can be grouped irrespective of range; thus in Fig. 2 the results are grouped in classes of 5% R.H. above 60% R.H., where more experiments were done, and in classes of 10% R.H. below 60% R.H. Each point indicates the mean intensity of



*a*

*b*

Fig. 2. Results shown in Fig. 1 grouped according to the highest humidity available in the chamber and irrespective of the difference of humidity available. *a* intensity of reaction expressed as a ratio  $D/W$ . *b*, intensity of reaction expressed as excess percentage on the dry side  $\left(\frac{100(D-W)}{D+W}\right)$ .

reaction for all the experiments in that class, plotted against the mean highest humidity available. Data obtained from some experiments at a higher temperature are also shown in this figure and will be referred to later.

When the data of Fig. 1 are plotted against the lowest humidity available instead of against the highest, there is a considerably greater scatter of points, indicating a less exact correlation.

The reaction increases as the highest humidity available approaches saturation. This is very similar to the results obtained by Thomson (1938) for the mosquito, but is in direct contrast to that of Kennedy (1937) for the locust. Kennedy found that the range available determined the intensity of reaction and that the specific values of the humidities were unimportant. Gunn (1937) found both factors of importance in the wood-louse.

#### THE INTENSE REACTION

The intense reaction at the moist end of the humidity scale can be made use of in investigating the location of humidity receptors (Pielou, 1940) and the mechanism of the humidity reaction (Gunn & Pielou, 1940). It was therefore examined in greater detail and was found to be very consistent as well as very intense. A standard gradient was adopted in which the controlling fluids were pure water and a sulphuric acid solution with an equilibrium vapour pressure giving a relative humidity of 90% at 25° C. (18.5% sulphuric acid by weight at 25° C.; Wilson, 1921). These fluids gave a gradient extending from very nearly 100% R.H. down to 94% R.H. in the standard 22 cm. chamber used in this laboratory. In twenty-nine experiments involving 145 animals and giving 5800 position records, 90.2% (S.D. 4.35%) of the position records were from the drier side at 94–97% R.H., 6.1% (S.D. 2.45%) from the moist side at 97–100% R.H., and the rest (3.7%) from the middle zone. This gives the high excess percentage on the drier side of 84.1% (i.e. 90.2–6.1), corresponding to a  $D/W$  ratio of 14.8. These data are shown graphically in Fig. 3.

In a series of six experiments in which the development of this reaction was recorded quantitatively, the reaction started very rapidly and increased progressively more slowly until after 15 min. it had practically developed its maximum value (Fig. 4).

#### EFFECT OF TEMPERATURE ON THE REACTION

A series of experiments, covering the entire range of humidity, was carried out in a constant temperature room at  $32.5 \pm 0.5^\circ$  C. The results obtained were almost identical with those in Fig. 1 and the data are grouped and shown in Fig. 2, along with the results of experiments at 25° C. The very close similarity of the two sets of points suggests that the animals react to relative humidity rather than to saturation deficiency. When the data for the two temperatures are plotted against saturation deficiency instead of relative humidity, there is a fairly marked distinction between the two sets of points, thus showing a less exact correlation. Thomson (1938) found

that the results for *Culex fatigans* also showed a closer correlation with relative humidity than with saturation deficiency. This conclusion is interesting in view of the stress that has been laid on the importance of using saturation deficiency instead of relative humidity in physiological work on the evaporation of water from insects.

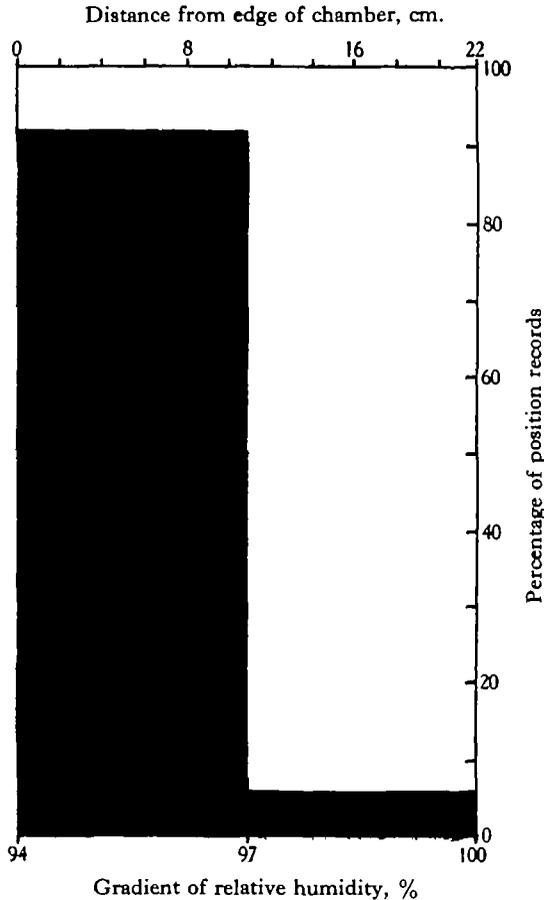


Fig. 3. Showing the marked reaction of *Tenebrio molitor* in an alternative chamber with a humidity gradient extending from 94 to 100% R.H. (*standard gradient*).

An attempt was made to perform a third series of experiments at a lower temperature (14° C.). At this temperature, however, the animals (which had been reared at 25° C.) were very inactive, very slow when they did move, and stiff and awkward in their movements; but they did, nevertheless, show the same general trend of results. The reaction was most intense when a very high humidity was available, but it was not nearly as intense as at higher temperatures. The efficiency of locomotion of these animals was obviously impaired by the cold, so this series of experiments was discontinued.

## EXPERIMENTS ON SINGLE ANIMALS

In the experiments described above, five animals were used at a time and their distribution was noted periodically, 15 min. after activating them mechanically. Another series was carried out, using one animal at a time in the standard gradient. Here the movements of the animal were recorded on squared paper and, with the aid of an audible time signal, half-minutes were marked on the tracks. From these drawings, the time spent in each half of the chamber was found. Altogether

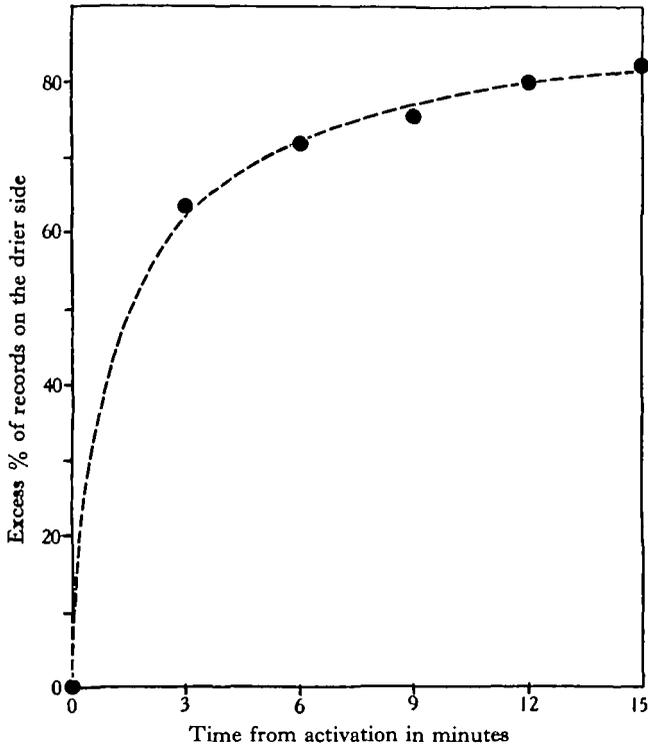


Fig. 4. The development of the reaction in the standard gradient. The reaction is practically fully developed after 15 min.

twenty-two such experiments were carried out, covering slightly over  $25\frac{1}{2}$  hr. Of this time, 24 hr. 32 min. was spent by the animals in the drier side and in 1 hr. 2 min. in the moister side. This gives an excess percentage on the dry side (time, not position records) of 92 %, compared with 84 % in the main experiments. In these experiments, the animals were not activated mechanically every 15 min. as they were in the main experiments. The slightly more intense reaction shown is, presumably, partly due to some slight increase in intensity after the first 15 min. (cf. Fig. 4).

## SUMMARY

1. *Tenebrio molitor* adults show a behaviour reaction to humidity. When normal undesiccated specimens are offered a range of humidities, they tend to collect in the drier region.

2. When the highest relative humidity available is not above 70% R.H., the reaction is slight but real. In a series of tests, as the highest humidity available approaches saturation, the intensity of reaction increases rapidly. It becomes nearly as intense as possible when the moistest region in the gradient approaches 100% R.H.

3. The difference between the two extreme humidities available—or the steepness of the gradient of humidity—is unimportant in determining the intensity of reaction. It is the value of the highest humidity that matters.

4. The intense reaction shown when the upper humidity approaches saturation is very consistent; it reaches its maximum value after about 15 min.

5. The behaviour of these insects appears to be more closely correlated with relative humidity than with saturation deficiency.

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