59.11, 53.2 : 57.72D

Article XV. — EFFECT OF HUMIDITY ON PUPAL DURATION AND ON PUPAL MORTALITY OF *DROSOPHILA AMPELOPHILA* LOEW.

By Adolph Elwyn.

The following experiments were undertaken at the suggestion of Dr. F. E. Lutz and Prof. R. W. Tower for the purpose of determining the influence of relative humidity, as an isolated factor, on the pupal duration and pupal mortality of the fruit fly, *Drosophila ampelophila* Loew.

To do this efficiently, it is necessary to devise a method for the adequate control and measurement of humidity, since in studying moisture phenomena, three conditions must be fulfilled:—

1. The production and sustained constancy of a desired humidity;
2. The accurate measurement of such humidity;
3. The isolation of humidity from other environmental factors, such as temperature, so as to assure the full and independent effect of the former.

The last condition is easily met. Attention, therefore, was wholly devoted to a satisfactory solution of the first two, a difficulty which in all probability has been the chief cause of the neglect of the humidity factor in environmental experiments.

Two series of experiments were made. The first was an attempt to determine roughly the direction of the effects, if any, of differences in relative humidity. In the second set, strictly controlled conditions were introduced to study the corresponding effects more exactly and conclusively. The strain of Drosophila was supplied by Dr. Lutz. The larvæ were reared in a glass box with side doors, and the newly formed pupæ were collected twice a day and immediately subjected to experimental conditions.[1]

Series I.— This series involved 426 pupæ placed on card-board and suspended in bottles stoppered with cotton under the following conditions:

A — Over water ..... 147 pupæ
B — Empty bottle, hence room humidity ..... 217 pupæ
C — Over calcium chloride ($CaCl_2$) ..... 62 pupæ

Daily tests made with a wet-and-dry-bulb hygrometer gave A = 100 per cent, B = 38–42 per cent (room humidity). C was not measured but was obviously of a low relative humidity. The average temperature was 61°–64° F.

[1] Lutz, F. E., Humidity — A Neglected Factor in Environmental Work. American Naturalist, XLVIII, pp. 122–128, 1914.

It may be said here that the wet-and-dry-bulb hygrometer was found altogether inadequate for the measurement of humidity in small vessels. Air which had been repeatedly passed through concentrated H_2SO_4 and over solid KOH, registered as high as 31 per cent when it was undoubtedly dry, and found to be so experimentally by the application of the dew-point apparatus used in series II. This failure of more exact registry is due chiefly to the practical awkwardness of regulating the amount of water necessary for the wet bulb, and of obtaining the passage of a sufficiently swift current of air through the small jars used. In series II, therefore, an apparatus was devised which completely eliminated the use of the wet-and-dry-bulb hygrometer.

Of the 426 pupæ, 383 developed into adults and the length of pupal period is shown in Table I.

Table I.

	Number of Pupae emerged			Percentage of Pupæ Emerged		
No. of days	A	B	C	A	B	C
7	0	1	0		.5	
8	1	1	0	.7	.5	
9	13	12	7	9.0	5.5	11
10	27	28	8	18.4	13.0	13.0
11	36	47	13	24.4	21.6	21.0
12	45	77	11	30.6	35.5	18.0
13	20	28	5	13.6	13.0	8.0
14	0	2	0		.9	
15	0	0	0			
16	0	1	0		.5	
Total	142	197	44	96.7	91	71

Empirical mode of A — 12 days.
" " " B — 12 days.
" " " C — 12 days.

On the surface there is a slight variation in mode in C. But the number used in C was very small, and a statistical examination considering the probable error, shows the variation to be negligible.

The effects on mortality were, however, more striking, as seen in the following table:

Table II.

Experiment	Per cent of Mortality
A	3.3
B	9.0
C	29.0

Here we have a distinct increase in mortality with each corresponding decrease in humidity. Roughly, the effects of changes in relative humidity on length of pupal period were negligible, while the effect on mortality was striking and definite.

SERIES II.— To determine accurately the effect of various relative humidities on the pupal duration and pupal mortality of Drosophila ampelophila, under strictly controlled conditions. Conditions:—

1. The accurate measurement of a given humidity.

Owing to the inadequacy of the wet-and-dry-bulb hygrometer (see above) a new apparatus was devised. This was a modified form of the Regnault dew-point apparatus, similar to the one employed by the U. S. Weather Bureau for determining humidities at high temperature. As shown in the diagram, it consists of a well polished silver tube (a), cemented to a longer glass tube (b) of the same diameter. The cork closing the top is provided with a glass tube (c) going to the bottom of the silver tube and connected with an aspirating apparatus (d); an outlet tube (e), and a delicate thermometer reaching to the center of the silver tube. The whole apparatus slips into a rubber stopper (f) which closes the mouth of the vessel whose humidity is to be determined. To make observations, the silver cup is filled with a volatile fluid, such as sulfuric ether, and the fluid evaporated by means of the constant pressure aspirator until the dew is just deposited, when a reading of the temperature is taken. This is the temperature of the dew-point, and the relative humidity of the atmosphere in the vessel can be found by dividing the vapor pressure at the temperature of the dew-point by the vapor pressure at air temperature. So for instance, if the vapor pressure at dew-point temperature is .0979 and that at air temperature is .595, then the relative humidity to be determined will be $\frac{.0979}{.595} = 16+$ per cent. For purposes of exactness the silver tube should be permitted to warm up, and readings repeated several times. If the tube is well polished and the observer has had good practice in distinguishing

faint depositions of dew, the results will be accurate and consistent and the error negligible.

2. Obtaining and keeping constant a desirable humidity.

All the experiments were conducted in closed vessels to facilitate the constancy of a produced humidity. By introducing respectively dry $CaCl_2$ and water, corresponding relative humidities of 0 per cent and 100 per cent were produced, these humidities being measured by the dew-point

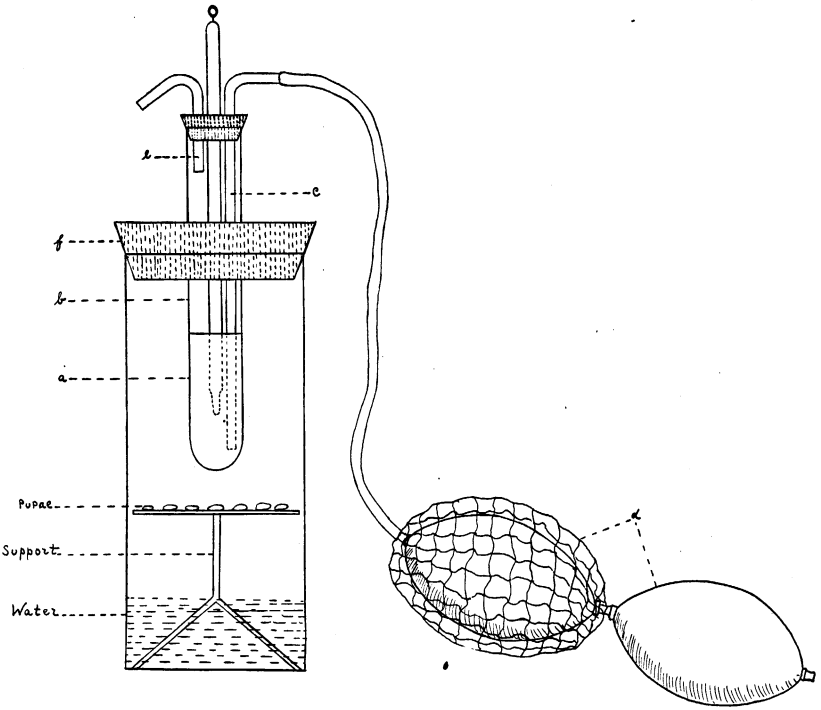

FIG. 1. Dew-point Apparatus.

apparatus. It was desirable, therefore, to obtain some constant intermediate humidity, say 50 per cent or 60 per cent. Now, saturated salt solutions in closed vessels have a definite vapor pressure, varying with the nature of the salt, and for this reason should give corresponding humidities. Vapor pressures have been experimentally determined for many salts, hence, if the proper salt were selected, the desired humidity would be obtained. For our purpose, the most convenient salt was sodium nitrate ($Na\,NO_3$), which has a vapor pressure .4393 at 68° F, hence a relative humidity of 64 per cent at that temperature. The numerous determinations with the dew-point

apparatus gave humidities never less than 61 nor more than 66 per cent. We therefore had three humidities to work with: 100 per cent, 61–66 per cent, and 0 per cent.

The experiments included 1137 pupæ distributed as follows, the average temperature being 65–66½° F.

Table III.

Per cent of Humidity	Number of pupæ
100	401
61–66	333
0	403
Total	1137

Of these 1137 pupæ, 873 developed into adults, and the pupal duration as well as the number emerged is shown in Table 4.

Table IV.

	Number of pupæ emerged			Percentage of pupæ emerged		
No. of Days	100%	68–66%	0%	199	61–66	0
5	0	0	1			.2
6	0	1	1		.3	.2
7	79	55	19	19.7	16.5	4.7
8	205	174	110	51.1	52.3	27.3
9	100	51	51	24.9	15.3	12.7
10	5	10	7	1.3	3.	1.7
11	2	1	1	.5	.3	.2
Total	391	292	190	97.5	87.7	47.0

Empirical mode of 100 per cent, 8
Empirical mode of 61–66 per cent, 8
Empirical mode of 0 per cent, 8

Thus there seems to be no obvious effect on the length of the pupal period, the variations being negligible even under close statistical scrutiny.

On the other hand the influence of changes in relative humidity on mortality is sharply defined, the experiments affirming the results of Series I.

Table V.

Per cent of Humidity	Per cent of Mortality
100	2.5
61–66	12.3
0	52.9

There is therefore no optimum humidity between 0 and 100 per cent which might be called normal, such as W. L. Tower [1] has found with *Leptinotarsa*, but there is a definite decrease in mortality with each corresponding increase of humidity, the optimum being a humidity of 100 per cent, when mortality is so low as to be almost negligible. When we group the pupæ in two divisions, according to their age, a very interesting fact appears. The following table shows a comparison of the total number with that of the two age groups. Of the 1137 pupæ, 366 were 2–8 hours old and 771 were 8–14 hours old, when subjected to experimental conditions.

Table VI.

Comparative Mortality.

	Per cent of Humidity	Per cent of Mortality total number	Per cent of Mortality Age 2–8 Hours	Per cent of Mortality Age 8–14 hours
A	100	2.5	2.2	2.7
B	61–66	12.3	27.3	9.4
C	0	52.9	77.3	34.6

While age is thus of but little consequence in medium A, it nevertheless greatly modifies the action of humidity in the other two media. B shows 27 per cent of mortality for young pupæ and only 9.4 per cent for the older

[1] W. L. Tower, — Evolution in Chrysomelid Beetles of the Genus Leptinotarsa. Carnegie Institution of Washington, 1906, Publ. No. 48.

ones; while C gives as high a mortality as 77.3 per cent for young as compared with 34 per cent for older pupæ. These facts are explained by the integumental changes which take place during early pupal life. When first formed the pupa has the pale yellow delicate transparent skin of the larva, and in this stage diffusion probably takes place rapidly. Hence a lowering of humidity would cause a quick evaporation of the pupal moisture with resulting death. But in a few hours the skin becomes brownish, more opaque and chitinized, so that evaporation of moisture takes place with more difficulty. When pupæ of this stage are placed in media of low relative humidity, much of their moisture is retained, and the effects are consequently greatly diminished. It is very likely that a relative humidity of 0 would cause 100 per cent mortality if the pupæ were subjected to such condition immediately after formation.

Summary.

1. Changes in relative humidity have no marked effect on the length of pupal period of *Drosophila ampelophila.*
2. Changes in relative humidity produce striking changes in the mortality of *Drosophila,* the mortality increasing with a decrease of humidity. The optimum humidity being 100 per cent.
3. The effects of low humidity on mortality are most marked with very young pupæ, whose covering permits a rapid evaporation of body moisture. After a few hours, when integumental changes making evaporation more difficult have set in, the effects of low humidity are correspondingly decreased.