

STUDIES ON THE BAR SERIES OF DROSOPHILA V.
THE EFFECTS OF REDUCED ATMOSPHERIC
PRESSURE AND OXYGEN ON FACET NUMBER
IN BAR-EYED DROSOPHILA

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THE investigations which have been directed toward a clarification of the kinetics of facet determination in the Bar series of alleles in *Drosophila* have, heretofore, been concerned largely with the use of temperature as a suitable and easily controlled variable. The manner in which temperature data have been used is clearly set forth in the work of a number of investigators, notably, KRAFKA, 1920; ZELENY, 1923; DRIVER, 1926; 1931; LUCE, 1931; HERSH, 1934; and MARGOLIS, 1935 a and b.

The present paper is the outgrowth of an effort on the part of the author to find other environmental agencies which might be brought to bear upon the problem. The data presented here deal with the effects of reduced atmospheric pressure, and of a pure oxygen atmosphere on the number of facets in the compound eye.

EXPERIMENTAL

The Effect of Reduced Atmospheric Pressure. The stock used in this phase of the work was a very homogeneous Bar stock designated as stock "B" in earlier studies (MARGOLIS 1936). The flies were reared in one by four inch shell vials containing a banana-agar medium (2 percent agar-agar), seeded with a concentrated yeast suspension (1 cake of yeast per 100 cc water).

A one hour egg-laying period was used in the experiments and the vials containing eggs were then segregated into suitable groups for purposes of the experiment. One group of vials was placed in an incubator at 28°C. for the total period of development; the second group was placed in a low pressure chamber adjusted to maintain a pressure of $\frac{1}{2}$ atmosphere (380-400 mm Hg); and the third group of vials was kept at atmospheric pressure very near to the low pressure chamber. The apparatus used for maintaining reduced pressures has been described fully by DUBIN (1934).

Neither the low pressure chamber nor the room in which it was situated was thermostatically controlled. The experiments were therefore designed to maintain the flies at reduced atmospheric pressure only during that period of development prior to the temperature-effective-period, that is, that period during which temperature is capable of affecting facet number. A preliminary determination of the temperature-effective period for facet

formation in the stock used indicated that the period begins during the second half of the egg-larval stage. This is substantially consistent with investigations on other Bar stocks (DRIVER 1931; MARGOLIS 1935b).

During the time that the flies were exposed to reduced pressure a temperature record of both the room and the low pressure chamber was kept. Frequent readings of the room temperature were made daily. In the case of the pressure apparatus it was not possible to take temperature readings while the chamber was in operation. The chamber was therefore opened for a short period daily and the temperature fluctuation for a 24 hour interval was recorded from a maximum-minimum thermometer. Table 1 gives the data on temperature control. The average temperature of the tank for a 24 hour interval was computed simply as the mean between the maximum and the minimum temperature for that 24 hour interval. It is evident that the average temperature calculated in this way may be subject to considerable error although the close approximations of chamber temperature to room temperature indicate that in this case there is probably no great error. The total range of temperature variation in the tank was 14.4° to 19.4° and in the room 15° to 19°.

TABLE 1
Temperature control in pressure experiment.

	AVERAGE ROOM TEMP.	AVERAGE TANK TEMP.
12/6	18.4°	18.1°
12/7	17.6°	16.7°
12/8	16.6°	16.6°
12/9	17.7°	17.5°
12/10	16.3°	16.7°
	17.3°	17.1°

After remaining at reduced pressure for 52, 74, 96, and 120 hours, respectively, groups of vials were transferred from the low pressure chamber to a 28° incubator. At these same times vials containing larvae which had developed at atmospheric pressure near the chamber were transferred to the same incubator to serve as controls. This incubator also contained those flies which had spent their full period of development there.

The data on facet number for the 3 groups of flies are given in table 2. The mean facet numbers for the flies reared at 28° for the total period of development serve as controls for both of the other groups, namely those flies spending a portion of their early development in the reduced pressure chamber before transfer to 28°, and those reared for corresponding periods at room temperature and atmospheric pressure prior to transfer. It is evident that neither of these groups had yet been affected by the lower temperature of the room or pressure chamber.

Our interest, however, centers upon the possible effect of the reduced pressure upon the mean facet numbers. Comparing, group for group, those flies which had spent part of their development at reduced pressure with those flies which had spent equivalent parts of their development at atmospheric pressure, a definite interpretation of the data is difficult. In the males there is evident in each group a small, but possibly significant, decrease in facet number in those flies developing at reduced pressure. When analyzed in terms of significance of differences we find that only in the 120 hour groups is the difference in means statistically significant. In the other three groups the difference in means is in each case less than twice the standard error of the difference.

TABLE 2
Effect of reduced atmospheric pressure on facet number.

HRS. AT ATMOSPHERIC PRESSURE BEFORE TRANSFER TO 28°	HRS. AT REDUCED PRESSURE BEFORE TRANSFER TO 28°	MALES		FEMALES	
		M ± S.E.	V PERCENT N	M ± S.E.	V PERCENT N
	52	66.8 ± 0.68	8.8—75	53.7 ± 0.80	11.4—58
52		68.3 ± 0.76	9.7—75	52.4 ± 0.69	9.6—53
	74	69.9 ± 0.89	10.9—74	51.0 ± 0.66	8.7—45
74		72.4 ± 0.97	11.5—74	53.4 ± 0.62	8.4—53
	96	69.4 ± 1.03	9.5—41	53.6 ± 0.75	8.7—39
96		71.6 ± 1.04	12.4—73	57.0 ± 0.89	10.9—49
	120	66.1 ± 0.81	10.0—67	53.6 ± 0.59	9.6—75
120		72.1 ± 0.96	8.9—45	55.4 ± 0.75	8.0—35
Controls at 28° for total development		71.6 ± 0.82	11.5—104	52.9 ± 0.45	8.3—96

In the females there is a similar but less systematic difference in mean facet numbers of corresponding groups. The flies transferred at 52 hours present the exception in that those which developed at reduced pressure are slightly, but not significantly, larger than the ones which developed at atmospheric pressure. The differences within the 74 and 96 hour groups, respectively, are statistically significant but not in the 120 hour group.

A definite conclusion concerning the effect of reduced atmospheric pressure on facet number is hardly justified beyond pointing out that there is a small but doubtfully significant reduction in number. We shall return to this after considering the effect of a pure oxygen atmosphere on facet number.

One other feature of the data in table 2 requires brief comment. The coefficient of variability for each group is given. In general there is no evidence of a trend in this statistic in groups transferred at successive intervals. This is further confirmation that the flies had not yet entered the temperature-effective period for the character under consideration (MAR-

GOLIS 1935 b; CHILD 1935). Moreover, there is no large or systematic difference between corresponding groups. This shows that the reduced pressure had not affected variability.

The Effect of a Pure Oxygen Atmosphere. The experiments to be discussed here were carried out about a year and a half after the experiments described in the preceding section. The same Bar stock (stock B) was used but this had been inbred for another 20 generations in the interval between experiments. This fact is pointed out since there is evidence of accumulation of modifiers for high facet number during the period of inbreeding. The facet counts in control flies reared at 28°, therefore, show a significant increase in the oxygen experiments.

TABLE 3
Effect of oxygen on facet number.

	MALES		FEMALES	
	M ± S.E.	V PERCENT N	M ± S.E.	V PERCENT N
Controls at 28°	80.7 ± 2.01	12.7—26	57.2 ± 0.91	10.1—40
Flies placed in O ₂ at 24 hours age for 48 hours	111.1 ± 3.01	14.3—28	76.6 ± 1.74	11.8—27
Flies placed in O ₂ at 48 hours age for 42 hours	101.3 ± 3.42	15.8—22	64.3 ± 1.33	11.0—28

In these experiments small vials of about 2 to 3 cc capacity were used for the development of the larvae. These vials were half-filled with the usual banana-agar medium seeded with a drop of thick yeast suspension. Ten to 15 eggs were placed in each vial which was then covered by two thicknesses of cheesecloth held in place by rubber bands. All vials were then placed in a 28° incubator to begin their development.

After 24 hours of development at 28° under ordinary atmospheric conditions, one group of vials was placed in 8 ounce bottles (one vial per bottle), into which a pure oxygen atmosphere was introduced. These bottles served as oxygen chambers and were immediately placed in the 28° incubator. The larvae were removed from the oxygen chambers after 48 hours and completed their development under ordinary atmospheric conditions at 28°.

The same procedure was followed with a second group of larvae after 48 hours of development under ordinary atmospheric conditions at 28°. This group remained in the oxygen chambers for only 42 hours.

The third group of larvae developed completely under ordinary atmospheric conditions at 28°.

The mean facet counts and coefficients of variability for the 3 groups of flies are given in table 3. The difference in means is clearly evident and statistically significant in both males and females. Flies developing from the 24 hour larvae exposed to oxygen for 48 hours show an increase in facet number over the controls of about 38 percent in the males and 25 percent in the females. Flies developing from the 48 hour larvae exposed to oxygen for 42 hours show an increase in facet number over the controls of about 25 percent in the males and 12 percent in the females.

In respect to the coefficients of variability there appears to be some slight increase in the males exposed to oxygen. This is of doubtful significance when the relative uniformity in the females is considered.

DISCUSSION

The use of temperature in earlier studies has made it possible to single out certain facet forming processes in virtue of their differential temperature characteristics, for example, those facet-forming processes which during a particular developmental period differ from other processes in their response to temperature. This period has been variously designated as the temperature-effective period or sensitive period. In the case of Bar this period falls approximately within the third quarter of egg-larval development, although there is some variation with temperature (DRIVER 1931).

It is evident that other processes determining facet number must occur in the interval between the initiation of development and the beginning of the temperature-effective period. It has not, however, been possible to single out these processes through the use of temperature in Bar. In the wild type, on the other hand, the effect of temperature on facet number extends back into early embryonic stages, possibly to the very beginning of development (MARGOLIS and ROBERTSON 1937).

The data on the effect of oxygen offer clear evidence that processes which determine facet number in Bar are taking place at least as early as the second day of development at 28°, that is, about a day before the beginning of the temperature-effective period. This may be seen by comparing the facet counts of the two groups of flies which were exposed to oxygen and also their periods of exposure. The group placed in oxygen at 24 hours of development showed the greater increase. This could only occur if facet determining processes were taking place some time before 48 hours of development. The time difference between the 48 hour and 42 hour exposures to O₂ is probably of no significance since in the flies exposed for the shorter period puparium formation had already begun, and at this time the ommatidia are established as morphological units whose number is already determined (KRAFKA 1924).

The preliminary character of the foregoing experiments leaves much to

be desired in the way of localizing the period during which oxygen affects facet number, which might be called the oxygen-sensitive period. Moreover, data on the effect of different partial pressures of oxygen would be very desirable.

We may return now to the bearing of the oxygen experiments on the possible significance of the small decrease in facet number brought about by reduced atmospheric pressure. The writer is disposed to the view that the decrease in facet number is real and not merely an error of random sampling. This is based upon the consideration that decreasing the partial pressure of oxygen may be expected to decrease facet number, since an increase in partial pressure resulted in increased facet number. Such an assumption, however, requires reservations since we may be dealing with a threshold phenomenon in which the partial pressure of oxygen, within a limited range, has no effect in altering facet number. Moreover, the pressure and oxygen experiments are not strictly comparable since in the former both the partial pressure of oxygen and total atmospheric pressure are reduced. The present experiments do not permit an assignment of the observed effect to one of these variables rather than the other.

While the data presented here are not adequate for answering many questions which arise, their significance is clear. The use of pressure and oxygen tension as environmental variables presents the possibility of further clarification of the kinetics of facet determination and may be applied to the study of other gene controlled developmental processes.

SUMMARY

Data on the effects of reduced atmospheric pressure and of a pure oxygen atmosphere on the development of facets in Bar-eyed *Drosophila* are presented.

Flies which had spent varying portions of their egg-larval period (2 to 5 days) at one-half atmosphere pressure showed a very small, but for the most part statistically insignificant decrease in facet number. Exposures to reduced pressure all took place before the beginning of the temperature-effective period.

Flies developed from larvae which had spent from the 24th to the 72nd hour of development in a pure oxygen atmosphere at 28° showed a marked increase in facet number when compared with controls raised under normal atmospheric conditions at 28°. A second group which had spent from the 48th hour of development to the 90th hour in oxygen showed a somewhat smaller but significant increase in facet number.

It is concluded from these results that facet determining processes are in operation before the beginning of the temperature-effective period, that is, about 50 hours at 28°; and that these can best be studied through the

systematic use of new and controllable environmental variables such as the ones used here.

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