THE DEVELOPMENT OF VESTIGIAL WINGS UNDER HIGH TEMPERATURE IN DROSOPHILA MELANOGASTER

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INTRODUCTION

THE PRESENT paper deals with three questions concerning the development of vestigial wings in Drosophila: First, where is the critical period during which the development of vestigial can be affected by high temperature (31°), the so-called temperature-effective period? Second, to what extent can the wing form be modified by temperature? And lastly, is the enlargement of the wing as a result of the temperature treatment due to the increase of size or number of cells?

The work of HARNLY (1930, 1932 and 1933), STANLEY (1928, 1931 and 1935), HERSH (1932) and others has already covered a considerable amount of ground in the study of the vestigial and temperature relationship in Drosophila. The present work, however, represents the beginning of a slightly different approach to the question and is concerned with a more direct embryological study than has hitherto been attempted.

THE TEMPERATURE-EFFECTIVE PERIOD

A pure vestigial stock of *Drosophila melanogaster* originally obtained from Columbia University, New York City, was used for the following experiments. The food was prepared from local material according to the formula adopted for this laboratory (LI 1930). Only two temperatures were employed to treat the vestigial flies: 25° and 31°C. For the lower temperature, we have used the cabinet gas-burning type of incubator designed by BRIDGES and LI (1932); for the high temperature, the only incubator available was of the water-bath type. During the years 1932 to 1934 the following experiments were performed.

High temperature treatment of pupae

In a number of cultures five pairs of strong and vigorous vestigial flies were allowed to breed. The larvae were raised in the 25° incubator and, as soon as puparia were formed, they were isolated within an hour. About 30 pupae thus isolated were put on a strip of moistened blotting paper and raised through the rest of their life cycle in a vial with a small amount of food to keep the paper continuously moist. These vials were then divided

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into two batches: "A" and "B." The one-hour old pupae in the vials of "A" series were allowed to start their development at 31°, but at each of the succeeding 6-hour intervals, were transferred to 25° for the rest of their life cycle. In the vials of the "B" series the pupae were allowed to begin their development at 25° and were subsequently transferred at the same time intervals to 31°. The purpose of the reciprocal transfers in the experiment was to narrow down from both ends of the pupal period the temperature-effective period for the high temperature. As controls, four cultures were tested simultaneously with the others. First, both larvae and pupae were raised at 25° (X1, table 3); second, the larvae were raised at 25°, but the pupae at 31° (X2); third, both larvae and pupae were raised at 31° (Y1); and lastly, the larvae were raised at 31°, but the pupae at 25° (Y2).

When the adults emerged from the pupal cases, they were etherized and their wings were measured under a binocular microscope with a standard micrometer. As a rule only the right wing of each fly was measured. However, when the latter was injured or folded, the left wing was then taken as a substitute, provided both wings were of approximately equal lengths. The results of the experiment are summarized in the following tables:

TABLE 1

The mean lengths in mm of vestigial wings of flies beginning their pupal period at 31° and transferred to 25° at the intervals indicated.

HOURS AT 31°	♀♀		♂♂	
	M	N	M	N
6	0.81	19	0.74	19
12	0.77	36	0.69	20
18	0.70	33	0.69	21
24	0.73	19	0.63	17
30	0.73	27	0.67	19
36	0.74	24	0.66	27
42	0.75	28	0.69	37
48	0.76	17	0.68	36
54	0.80	26	0.70	25
60	0.75	18	0.70	26
66	0.79	27	0.68	24
72	0.73	24	0.66	27
78	0.77	30	0.74	27

The results given in tables 1 and 2 show that the mean lengths of the vestigial wings of the treated flies are fairly uniform, which means that there is no particular period in the whole pupal stage that responds to high temperature treatment. Comparing with the 25° control (X1, table 3), it can be further shown that there is no significant increase in wing length as a result of high temperature. If there is any effect at all, high tempera-

TABLE 2

The mean lengths in mm of vestigial wings of flies beginning their pupal period in 25° and transferred to 31° at the intervals indicated.

HOURS AT 25°	♀♀		♂♂	
	M	N	M	N
6	0.68	15	0.64	11
12	0.71	15	0.61	22
18	0.79	19	0.68	17
24	0.76	17	0.70	21
30	0.73	20	0.72	30
36	0.78	19	0.72	42
42	0.78	36	0.71	25
48	0.79	20	0.74	25
54	0.78	28	0.73	32
60	0.82	17	0.72	25
66	0.79	30	0.69	24
72	0.86	28	0.71	21
78	0.83	27	0.75	30

TABLE 3

The mean lengths in mm of wings of all vestigial flies raised in both "A" and "B" series and in the controls.

SERIES	♀♀		♂♂	
	M	N	M	N
"A"	0.75±0.01	328	0.69±0.01	325
"B"	0.78±0.01	291	0.71±0.01	325
X1	0.80±0.01	132	0.74±0.01	129
X2	0.78±0.01	12	0.71±0.01	15
Y1	1.06±0.04	32	1.63±0.05	25
Y2	1.05±0.01	124	1.45±0.02	122

ture tends to make the vestigial wings slightly shorter when the pupae are subjected to it, as indicated in table 3. When we compare the mean lengths of the vestigial wings of all the flies in both "A" and "*B*" series and those of the controls X1 and X2, it can be noted that when the pupae were raised at 31,° the wing length of the fly is consistently shorter than otherwise. The results in table 3 also show that when the larvae were raised at 31°, irrespective of treatment of the pupae, there is a great increase in wing length (Y1 and Y2, table 3). It follows therefore that a much more significant temperature-effective period is to be found in the larval period.

High temperature treatment of larvae

In order to locate this critical period in the larval stage by means of reciprocal transfers the larvae of various ages were exposed to 31°. This would naturally necessitate careful isolation of the newly hatched larvae,

as it has been shown that there is a considerable amount of variation in the time of hatching of eggs isolated at the same time (Li 1927). For this purpose a large number of vials was prepared, in each of which a glass slide with a piece of blotting paper of corresponding size was placed. The blotting paper was first soaked in fermented banana juice and then a thin layer of banana agar food was carefully spread on it. Five pairs of strong parental vestigial flies were allowed to remain on the food while eggs were laid during a 6-hour interval in the 25° incubator. Slides with eggs on them were taken out of the vials and examined one by one under a binocula. The newly hatched larvae within thé interval of one hour were isolated with a fine scalpel and transferred to a culture bottle, fifty larvae in each bottle. Thus they were all about one hour old and allowed to develop under almost identical conditions of food and amount of space. The culture bottles in duplicates were then treated as in the case of pupae in the earlier experiments, that is, reciprocal transfers between two temperatures 25° and 31°. As soon as the larvae pupated the bottles were all removed to 25° to let the flies finish the rest of the life cycle. Tables 4 and 5 give the results of the wing measurements of the treated flies.

In working through the data shown in the above two tables one may note that there is a great variability in the wing measurements of the individual flies as indicated by the high value of the coefficient of variabilities (C.V., tables 4 and 5). The percentages of mortality in most cases

TABLE 4

Mean lengths in mm of vestigial wings of flies beginning their larval period at 31° and transferred to 25° at the intervals indicated.

HOURS AT 31°	♀♀			♂♂			PERCENT MORTALITY
	M	C.V.	N	M	C.V.	N	
6	0.85±0.01	9.53	21	0.72±0.02	9.40	12	38.91
12	0.89±0.02	14.18	23	0.82±0.01	7.02	19	22.21
18	0.86±0.01	9.69	20	0.75±0.01	9.33	11	42.58
24	0.83±0.01	10.32	23	0.78±0.01	9.39	19	22.21
30	0.85±0.02	11.87	19	0.73±0.01	8.22	31	57.70
36	0.86±0.01	12.71	33	0.73±0.01	7.45	53	21.75
42	0.88±0.02	15.10	31	0.75±0.01	9.00	32	51.75
48	0.92±0.02	11.01	22	0.78±0.01	7.28	38	44.38
54	0.86±0.01	10.26	26	0.86±0.01	14.90	43	37.75
60	0.93±0.01	10.05	35	0.89±0.03	22.85	29	40.75
66	1.02±0.02	10.94	24	0.90±0.01	6.39	23	12.96
72	0.99±0.02	10.48	20	0.90±0.03	16.22	15	35.20
78	1.09±0.01	16.18	17	1.49±0.05	22.21	18	35.20
84	1.05±0.03	23.30	28	1.23±0.05	30.28	29	47.20
90	1.12±0.03	20.08	33	1.44±0.06	28.30	19	51.83
25° control	0.80±0.01	15.08	132	0.74±0.01	11.21	129	—
31° control	1.06±0.04	25.87	31	1.63±0.05	22.41	25	—

TABLE 5

Mean lengths in mm of vestigial wings of flies beginning their larval stage at 25° and transferred to 31° at the intervals indicated.

HOURS AT 25°	♀♀			♂♂			PERCENT MORTALITY
	M	C.V.	N	M	C.V.	N	
6	—	—	—	—	—	—	100.00
12	0.95±0.04	10.41	14	1.64±0.06	17.20	9	57.49
18	0.94±0.02	11.62	23	1.47±0.05	14.97	8	42.60
24	0.93±0.02	9.21	14	1.55±0.03	10.70	12	51.75
30	1.03±0.03	17.90	25	1.33±0.04	26.49	29	50.00
36	0.90±0.02	10.72	19	1.20±0.04	23.70	24	20.38
42	0.92±0.02	14.40	33	1.23±0.06	36.85	28	43.51
48	1.06±0.04	28.81	33	1.04±0.04	29.50	32	39.80
54	0.91±0.02	12.31	22	0.82±0.01	13.57	41	41.70
60	0.93±0.01	11.83	31	0.88±0.03	20.42	20	53.72
66	0.83±0.01	15.00	47	0.81±0.01	15.45	43	16.67
72	0.92±0.03	16.91	14	0.77±0.01	8.82	17	42.55
78	0.74±0.01	8.43	21	0.72±0.01	8.74	25	14.82
84	0.77±0.01	12.82	45	0.66±0.01	11.50	40	21.30
90	0.71±0.01	9.93	50	0.65±0.01	10.80	40	16.68
25° control	0.80±0.01	15.08	132	0.74±0.01	11.21	129	—
31° control	1.06±0.04	25.87	31	1.63±0.05	22.41	25	—

are very high, especially when the larvae were first started in 25° and later transferred to 31°. These coupled with the small number of individuals measured made it hazardous to draw any definite conclusions as to the effect of high temperature on the wing development of the vestigial flies. However, as indicators of the time at which high temperature begins to exert its influence on the development of vestigial wings and when this influence ceases, the data do show certain things. In cases where the larvae started their development at 31° and then were transferred to 25° (table 4), it can be noticed that at about the sixtieth hour there is a decisive tendency for the vestigial wings to become longer. This point must be taken as the beginning of the temperature-effective period for 31°C. In the case of the opposite transfers (table 5) it is also easily seen that beginning with the seventy-eighth hour, the mean length of the vestigial wings becomes steadily shorter. This must mean that the temperature-effective period ends before the latter point is reached. On the basis of these observations we may fix the temperature-effective period for vestigial wings roughly at sixty to seventy-two hours in the larval period, making a total of approximately 12 hours. The data further show that at 31°, with the stock of vestigial flies used, there is a distinct sexual-dimorphism in the wing lengths such as observed by the earlier workers (ROBERTS, HARNLY and STANLEY).

In order to overcome some of the difficulties in variability and mortality encountered in the above experiments, a number of similar tests were made. In these later attempts, we used twice as many flies from a vestigial stock that had been inbred for nine generations. Another deviation in procedure from the earlier experiments was that after they had been isolated the larvae were allowed to develop for the first 24 hours at 25° without any disturbance, at the end of which, they were all transferred to 31°, except of course the 25° controls. From 31° at each of the succeeding 6-hour intervals, the larvae were again transferred to 25° to complete their development. No transfers in the opposite direction were made.

The reason for allowing all the larvae to pass the first 24 hours in 25° instead of 31° is primarily an attempt to reduce mortality of the larvae. Since the temperature-effective period is found to be approximately in the latter half of larval life, the elimination of the first day of high temperature treatment should not interfere with the result as far as the location of the critical period is concerned. At the same time, it may be of interest to see whether such elimination would affect the development of the vestigial wings. In order to test this point, two 31° controls were employed. In one of these, vestigial flies were raised throughout their larval stage at 31° (control 2, table 6), while in the other, the larvae were first exposed to 25° for one day like the rest of the cultures in the experiment and then were transferred to 31° (control 1, table 6).

Table 6 gives the results of one such experiment. It can be seen that

TABLE 6

Mean lengths in mm of vestigial wings of flies, kept at 25° for the first 24 hours of their larval period, raised at 31° and subsequently transferred to 25° again at the intervals indicated.

PERIOD AT 31°	♀♀			♂♂			PERCENT MORTALITY
	M	C.V.	N	M	C.V.	N	
24–30	0.81±0.01	11.09	67	0.74±0.01	12.12	56	38.5
24–36	0.81±0.01	12.59	86	0.73±0.01	15.92	88	13.0
24–42	0.79±0.01	9.38	85	0.74±0.01	9.37	69	23.0
24–48	0.81±0.01	10.62	81	0.73±0.01	8.87	76	21.5
24–54	0.80±0.01	9.13	81	0.74±0.01	9.36	73	23.0
24–60	0.85±0.01	12.05	65	0.84±0.01	6.02	62	36.5
24–66	1.02±0.03	21.60	87	1.07±0.04	29.03	89	12.0
24–72	1.07±0.03	19.82	75	1.16±0.04	21.85	64	30.5
24–78	1.01±0.02	13.61	69	1.16±0.04	24.42	57	37.0
24–84	0.92±0.02	13.12	64	0.99±0.03	25.30	78	29.0
24–90	0.99±0.02	15.16	54	0.99±0.04	26.50	65	15.5
25° cont.	0.79±0.01	13.12	77	0.72±0.01	8.94	98	12.5
31° cont. 1	1.03±0.04	25.32	81	1.28±0.04	26.10	84	17.5
31° cont. 2	1.73±0.05	30.40	74	1.77±0.05	21.01	32	47.0

beginning with the sixtieth hour of larval life, the lengths of the vestigial wings of the treated flies show a distinct increase, especially in the males. In the females the increase in lengths of wings is not very striking until the sixty-sixth hour interval. It is possible that by starting the larvae at 25° for the first day, the whole developmental process may be somewhat slowed down, as compared with those which were raised entirely at 31°, thereby causing a slight delay in the onset of the critical period. The slight drop in the mean wing lengths after the seventy-eighth hour interval may not be very significant. It should not be interpreted as the end point of the temperature-effective period; without data on the opposite transfers, such an end point cannot be determined with certainty.

Regarding the increase of wing length of the flies exposed to high temperature, one may note that the striking difference in wing lengths between the sexes is very much reduced if not entirely gone. This probably means that with inbred stock, more or less homogeneous in its make-up, the so-called sexual-dimorphism can be eliminated and in both sexes the development of vestigial wings then responds to high temperature equally well. However, when the first day of the larval period was not subjected to high temperature, neither sex of the vestigial flies could have wing lengths comparable to those whose whole larval period was subject to it. This is clearly shown in the two 31° controls (controls 1 and 2, table 6). So in order for the vestigial wings to reach the maximum length, the first 24 hours of the larvae must be treated with high temperature (in this case 31°). It follows therefore that high temperature at this particular period supplements the process involved in the enlargement of the wings. This supplementing process however could do nothing unless the larvae were continuously treated under high temperature during the critical period (60–72 hour interval).

The same point can be verified from yet another experiment. When only short intervals of 12 (60–72 hours) or 24 (48–72 hours) hours from the latter half of the larval period of the vestigial flies and covering the whole length of the temperature-effective period were subjected to 31° the average lengths of the wings of either sex could not be increased to more than 1.03 ± 0.01 mm (from unpublished data). In view of these facts, any assumption that the increase in size of the vestigial wings is due to high temperature treatment only at the critical period may be held as questionable.

THE FORMS OF THE WINGS OF THE TREATED VESTIGIAL FLIES

As we examined the wings of the treated vestigial flies at the time when they were measured, we noted that there was a great deal of variation in the forms of the wings. Greatest variation was found in flies longest ex-

posed to 31° and in the 31° control. After having made all the necessary measurements, we fixed these flies in 95 percent alcohol. Wings were taken off from the flies very carefully with fine forceps under a binocular and dehydrated by passing through the diaphane solvent and finally mounted with diaphane. Drawings were made under the Edinger Drawing apparatus according to the same scale of enlargement.

The regular vestigial flies are not capable of flying but those raised in high temperature may have wings enlarged enough to fly almost as well as the wild type. However, their wings are comparatively thinner than those of the wild types and therefore are easily folded and injured. Balloon formation by the separation of the upper and the lower surfaces of the wings either partly or completely was of common occurrence especially when the flies were fixed in weaker alcohol.

In the untreated vestigial wings (figures 1 and 2), it can be shown that except the marginals, all the longitudinal veins of a typical Drosophila wing are present and easily identified under a binocular microscope. A short axillary vein accompanied by a humeral crossvein is also present. The first three longitudinal veins are branched from the main stem which is divided very distinctly by two transverse sutures. The fourth, fifth and the smaller sixth longitudinal veins are developed very irregularly. The anterior and posterior crossveins together with anal crossvein are entirely absent. Among the vestigial flies the wing forms are not at all uniform, but as a rule the wings look folded and thickened, apparently due to the contraction of the longitudinal veins.

For the sake of description the various wing forms produced by high temperature treatment are conveniently classified into five types as follows:

Type 1 (figures 3 and 4): These are similar to the untreated regular vestigial wings. However by careful study, one may note four differences: First, the edges are more or less invaginated at the point where the longitudinal veins end; second, the posterior and anal crossveins are usually present; third, the marginal hairs are developed in places along the costal and the first longitudinal veins; fourth, the wings are slightly longer and broader than the untreated vestigials.

Type 2 (figures 5 to 14 inclusive): In type 2 there are two subdivisions (a) and (b), one of which is obviously derived from the other.

(a) The wings are longitudinally well extended but slender and pointed at the distal end. The marginal cell is present as a narrow strip. The anterior and anal crossveins are well developed; and the second longitudinal vein is usually fully extended sometimes even bearing marginal hairs. The latter may also develop at the region in front of the distal end of the first longitudinal vein (figures 5, 6 and 7).

(b) The wings are similar to (a) except the distal end of the wing turns upward and is often enlarged and somewhat rounded (figures 8, 9, 10 and 11).

Type 3 (figures 12, 13 and 14): The wings are broader than those of type 2 and the distal end is somewhat blunter and broader than the proximal end. The posterior crossvein is usually present or at least shows a rudimentary development.

Type 4 (figures 15 to 20 inclusive): Wings in this case show the marked development of the submarginal and the second posterior cells to present the general appearance of a fork. The posterior crossvein is always present. Sometimes the submarginal cell, the second posterior and the third posterior cell are especially extended to form two or three projections at the distal portion of the wing.

Type 5 (figures 21, 22 and 23): In this type of wing, all the typical wild-type characteristics are present with the one exception that the margin may be cut or notched in one or several places. The wings are usually attached to the body of the fly in the same manner as that of the wild-type.

It must be noted that while type 1 and type 5 described above undoubtedly represent two extremes and conceivably the latter might have been resulted by an all round expansion of the former, one cannot be sure that the intermediate types (types 2, 3 and 4) belong to a progressive series from the narrow to the broad types of wings. On the contrary, they may have occurred quite at random. The similarity of these forms to strap, antlered and various other allelomorphic phenotypes has already been pointed out by earlier workers (STANLEY 1931, etc.).

It is interesting to note that in the vestigial wings of the untreated flies, all the longitudinal veins of the wild-types are present. The enlargement of these vestigial wings as a result of temperature treatment is accompanied by the extension of these longitudinal veins. The posterior crossvein appears only when the wing reaches a certain breadth and the finishing touches of the wing development are the marginal hairs.

Besides the types mentioned, there are several other peculiarities observed, such as wings which appear as a triangle or like a tongue and sometimes an extra crossvein parallel with the anterior crossvein may be developed. Perhaps the most remarkable fact of all that we have observed is that in a number of cases the right and left wings of the same individual were strikingly different (figures 26 to 29 inclusive).

The size and number of cells of vestigial wings after high temperature treatment

Vestigial flies obtained from the experiment just described have furnished material for this study. The flies were first cooked with 10 percent

KOH and washed through a series of alcohols before the wings were taken off and mounted in diaphane, which would make the wing very transparent. For cell counting, we have adopted the method of DOBZHANSKY (1929). The work was done under the Edinger apparatus. The submarginal cell of the wing was arbitrarily chosen as the marked area for hair counting because of the several advantages. 1 The particular cell is present almost in all cases; 2 it is clearly defined by the second and the third longitudinal veins; 3 this particular cell is very sensitive to temperature treatment; and 4 the hairs here are comparatively regularly arranged and evenly distributed. Two kinds of counts were made: one on the hairs of the whole area of the cell and the other on those within a standardized small area inside of the cell. Specimens from ten of the males for each of the differently treated vestigial cultures were taken at random and thus examined. The results of these counts are shown in table 7.

TABLE 7

The average number of hairs in the submarginal cell etc. of the vestigial wings of flies raised at high temperature during the larval stage in the various periods indicated.

PERIODS EXPOSED TO 31°C.	NO. HAIRS IN SUBM. CELL	LIMITS	NO. HAIRS IN STANDARD AREA	LIMITS
24–30	135	40– 200	38.5	28–48
24–36	146	80– 250	37.8	28–43
24–42	246	90– 640	38.7	31–48
24–48	162	110– 250	36.7	31–41
24–54	214	130– 280	35.7	31–42
24–60	330	170– 580	32.7	25–39
24–66	799	400–1120	35.1	31–39
24–72	771	480–1100	32.1	26–38
24–78	827	600–1170	32.3	28–35
24–84	828	570–1020	30.6	24–35
31° cont.	1094	940–1200	30.2	28–32
wild-type ♂♂	1723	1680–1760	20.7	19–22

It is evident from the results shown in the above table that the number of cells in the submarginal cell was increased with the increasing length of exposure to high temperature, while a somewhat reversed situation is seen in the case of cell counts within the standardized area. There is also a wide range of variability in both counts, but the range gradually diminished as the flies were exposed to high temperature for longer periods. Comparing with the wild-type males, one finds that the average number of cells per chosen area is more constant and both the number and size of the cells in the submarginal cell of the wing are larger in the wild-type than any of the treated vestigials. While the increase in size of cells in

the wings of the treated vestigial flies may be due to a large extent to the stretching of the wings, the increase in number of cells must be the result of high temperature treatment. It is concluded therefore that the enlargement of the vestigial wings of the flies raised in high temperature is largely due to the increase in the number of cells.

Besides the experiment just described, we have also made a comparative study of the larval wing buds of (1) the vestigial flies (2) those under high temperature treatment and (3) the wild-type. As the results will be taken up in a separate paper, only a general statement will be made here. Beginning with the fifty-fourth hour of the larval period, the posterior part of the mesothoracic bud was seen to expand gradually. This change was more clearly seen in wild-type and in temperature-treated vestigials than in the untreated ones. From the sixtieth to seventy-second hour period the so-called wing bud begins to be formed from the posterior portion of the mesothoracic disc. The wing bud rudiments in wild-type and in treated vestigials are comparatively more pronounced than those of untreated vestigials. Comparing the time occupied by the temperature-effective period in the larval stage with that during which the changes of the imaginal discs occur, one may be led to conclude that high temperature at this critical period has an accelerating effect upon the growth of the mesothoracic bud and causes it to develop a larger wing bud in the vestigial flies.

DISCUSSION

It was ROBERTS (1918) who first discovered that there is a peculiar relation between high temperature and the development of the enlarged vestigial wings. He further showed that the critical period of temperature effect lies between the fertilization of the egg and the pupal stage. Among recent investigators along this line, HARNLY (1930, 1931 and 1933) and STANLEY (1928, 1931 and 1935) have done some very critical work. HARNLY (1930) found that there is a progressive increase in length of the vestigial wings with the progressive increase in temperature from 18° to 31°C. He later (1933) showed that the gradual enlargement of the vestigial wing under high temperature (30° 31° and 32°) follows a typical sigmoid growth curve and that the growth period extends from the sixtieth to eighty-fourth hour after the egg starts to develop.

Much detailed work has been done by STANLEY (1928 etc.) in locating the temperature effective period for such temperatures as 17°, 27°, 30° and 31° etc. Although both HARNLY and STANLEY started their experiments with only the age of eggs known, their data on the temperature-effective period for 31° are fairly comparable with what we found with the larva-isolation technique. It seems to us that the latter method of determining

the critical period has at least two advantages. In the first place, we can get a more exact knowledge of the mortality rate of the larvae and, secondly, a more accurate determination of the time interval in the larval period as such. According to our data, this critical period begins roughly at the sixtieth and ends at the seventy-second hour of the larval period. This particular period approximates to the time when the imaginal discs for wings are about to be differentiated from the mesothoracic buds (CHEN 1929).

It is possible, as shown in our tests, that high temperature in the pupal period may have some effect upon the size of the vestigial wing, causing slight reduction in the size (table 3). We have also found that the temperature in the first 24 hours of the larval life tends to accelerate the enlargement of the wings provided the flies are also exposed to it during the temperature-effective period. By raising the larvae from the beginning of the larval period at 31°, the vestigial wings may reach an average length of 1.73 ± 0.05 mm for the females and 1.77 ± 0.05 mm for the males. These figures are quite comparable to those which STANLEY found for the same temperature (STANLEY 1935). But when the larva was allowed to develop at 25° for the first 24 hours and then raised through the rest of the larval period at 31°, there was a considerable reduction in the mean lengths of the vestigial wings in both males (1.28 ± 0.04) and females (1.03 ± 0.04). It is of interest to note that according to CHEN (1929) the mesothoracic disc, from which the wing anlage is derived, appears during the first 24 hours of larval life.

The fact that different temperatures give different growth curves (HARNLY 1933) and that they have also different effective periods (STANLEY 1935) indicate that the situation is by no means a simple one. The complex situation is further shown by the high degree of variability in wing form. It becomes necessary that the whole problem should be looked into from a somewhat different angle. A study therefore from the embryological point of view may help to clear up some of the confusion. GOLDSCHMIDT (1935) in connection with his study of genes and external characters has examined the development of a series of mutant types affecting the form of the wing in Drosophila, such as vestigial-notch, cut, beadex, Beadex-Jollos and vestigial. By comparing the changes of the imaginal discs of the wings in the various mutations mentioned above in the pupal stage (from 8 to 30 hours), he found that although the wings of the adults of the above differ from one another, they all start in the early pupal period from imaginal discs comparable in size and pattern. Later however degeneration (Erkrankung) of the epithelial tissues of the wing buds sets in first from the tip, next the hind border and then the front margin, causing the wings to be remodeled according to the type of

genes affecting them. In the case of vestigial wing, the anlage according to GOLDSCHMIDT'S findings is actually 2 to 3 times as large as the adult wing before the degeneration process sets in and makes it shrivel to produce the adult pattern.

GOLDSCHMIDT'S study of the series of mutations from vestigial-notch to vestigial led him to infer that in the case of vestigial, the imaginal disc of the wing is somewhat comparable in size and pattern to that of the wild-type, but the degeneration of the epithelial tissue of the anlage starts much earlier (in the larval stage) than the others, such as cut, beadex and Beadex-Jollos, thereby giving rise to a much smaller and abnormal type of wing. Accordingly, he is inclined to interpret the temperature effect on the development of the vestigial wing in a different way from what has been implied by the earlier workers. According to GOLDSCHMIDT, the increase in size of the vestigial wing as a result of temperature treatment during the development of the fly is possibly due to the delay of the onset of the degeneration process rather than the direct increase in the size of the anlage.

Our work with the cell counts of the vestigial wings of flies raised under high temperature shows clearly that there is a decided increase in the number of cells. Unless the temperature effect is to prevent more cells from becoming degenerated, which is unlikely (since high temperature in the pupal period tends to decrease rather than increase the size of wings in the vestigial flies), it must be taken to mean that it has an accelerating effect in the growth period. It is unfortunate that GOLDSCHMIDT has not examined the imaginal discs of the vestigial flies in the larval period, so it is not possible to say, from his evidence to what extent the anlage of the vestigial wing is comparable to the other types. Our as yet unfinished work in connection with the comparative study of the imaginal discs of the mesothorax and the wing of larvae from wild-type, vestigial and vestigial raised at high temperature tends to show that these discs are larger in the case of the treated than the untreated vestigials. Without direct evidence at hand, we may have to infer that the more rapidly growing buds of the treated vestigials are the result of a higher rate of cell division in the formative period before the anlage starts to degenerate. These facts may somewhat modify the point of view held by GOLDSCHMIDT and they tend to show that the ultimate size and pattern of the vestigial wings of flies raised in high temperature is the result of at least two interrelated factors: the enlargement of the anlage of the mesothorax and wing on one hand and the delay of the onset of degeneration process on the other. Perhaps the high degree of variation in size, form and period of temperature effectiveness may all be explained when these two factors are carefully analyzed.

SUMMARY

1. Newly formed puparia of vestigial flies in Drosophila were isolated in vials and treated with the two temperatures 31°C and 25°C. The vials were divided into two series; the puparia in one of the series were first exposed to 31°C and then transferred in each of the succeeding six-hour intervals to 25°C until the end of the pupal stage; those of the other series were used as the opposite transfers.

2. The wing lengths of the vestigial flies so treated were measured and compared with the controls to see if the temperature had any effect on the flies. The results of the experiments tend to show that high temperature (31°C) is not effective in lengthening the wings of the vestigial in the pupal period. Possibly it may cause a slight reduction in the mean lengths of the wings.

3. However, when in like manner, the same temperatures were applied to the newly hatched larvae of the vestigial flies, the data on subsequent wing measurements showed that the high temperature was very effective in enlarging the vestigial wings. The temperature effective period is located at an interval which begins at approximately the sixtieth and ends at the seventy-second hour of the larval period.

4. By raising the first-day larvae in 25°C and then subjecting them to high temperature treatment described above, it was shown that the mean wing lengths of the vestigials so treated were significantly shorter than the cases when the first twenty-four hours of the larvae were also spent in high temperature. The evidence thus indicates that in order to realize the maximum lengthening of the wing characteristic of the temperature 31°C, not only the so-called temperature effective period, but also the first twenty-four hours of the larval stage must be exposed to the temperature.

5. The forms of the wings of the vestigial flies that have been exposed to 31°C for the whole (or almost whole) larval period showed a high degree of variability. This variation seems to bear no definite relationship with the sex of the individual or the size of the fly. Furthermore, the right and left wing of the same individual may occasionally be strikingly different.

6. By making various hair-counts of the submarginal cell of the wings of the treated flies, it is possible to show that the gradual enlargement of the wings as a result of high temperature treatment is primarily due to the increase in number rather than size of the cells.

7. The results of the above observations lend themselves to the interpretation that the high temperature tends to cause an increase in size of the mesothoracic and later the wing buds by increasing the rate of growth and cell division in these bodies and that the temperature seems to be particularly effective at the time when these buds start to form in the larval period of the fly.

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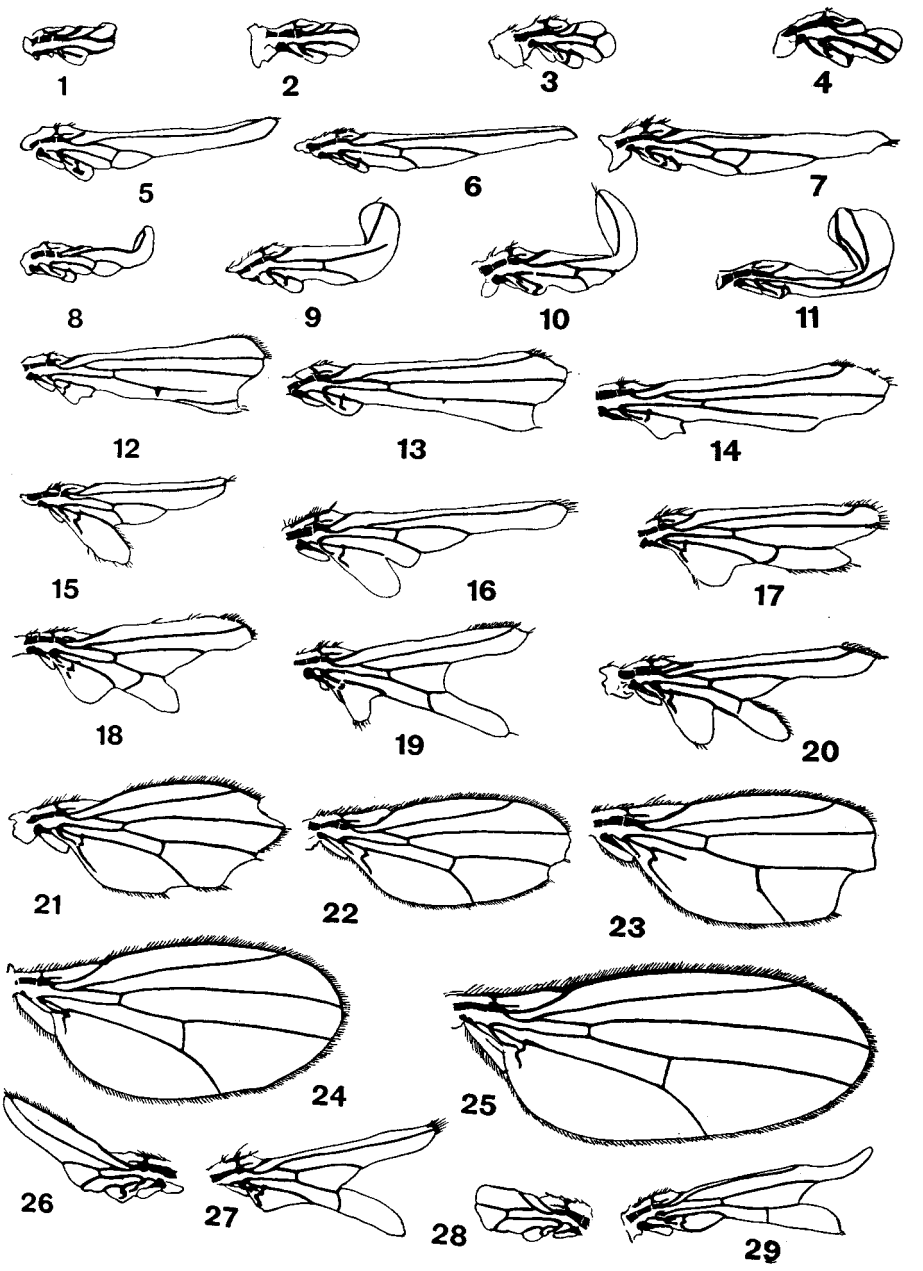

FIGURES 1, 2—Normal vestigial wings.
FIGURES 3, 4—Type 1 wing variations.
FIGURES 5–7—Type 2a wing variations.
FIGURES 8–11—Type 2b wing variations.
FIGURES 12–14—Type 3 wing variations.
FIGURES 15–20—Type 4 wing variations.
FIGURES 21–23—Type 5 wing variations.
FIGURES 24, 25—Wild type wings ♂ and ♀ respectively.
FIGURES 26, 27—Left and right wings in one individual.
FIGURES 28, 29—Left and right wings in another individual.