

THE TEMPERATURE-EFFECTIVE PERIODS AND
THE GROWTH CURVES FOR LENGTH AND
AREA OF THE VESTIGIAL WINGS OF
DROSOPHILA MELANOGASTER

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INTRODUCTION

ROBERTS (1918) first reported the increase in size of the wings of the vestigial mutant of *D. melanogaster* when reared at high temperatures and showed that the effect was more pronounced in the males than in the females. STANLEY (1928) stated that "the length of vestigial wing varies directly with the temperature, but not in direct proportion." I have shown (1930a) that there is a critical temperature for the increase in the length of the vestigial wings and that the marked change occurs in the females at a temperature 1° higher than in the males, a response showing sexual dimorphism. The curves for total development at one temperature of STANLEY (1931) and mine (1930a) were similar over the temperature range examined (17° to 31°). In preliminary reports (1930b, 1932, 1933) I have indicated that the temperature-effective period for increasing the size of the vestigial wings at 30°, 31°, 32°, and 33° C. is during the larval period commencing at approximately 60 hours and extending for various intervals with different maximum rates depending upon the sex of the individuals and the temperature experienced by the larvae. STANLEY (1931) found entirely different temperature-effective periods at 17° and 27° but in his recent paper (1935) he reports intervals at 30° and 31° easily harmonized with the periods reported here.

STOCKS AND METHODS

An inbred stock of vestigial winged flies should be homozygous for practically all the genes concerned in wing development and consequently the variability due to recombinations of modifying genes would be reduced to a minimum. Single pair brother-sister matings were made and selection was practiced in each generation, the sib matings being made from the bottle showing the best yield and with the flies showing the largest vestigial wings. The selection practiced should accelerate the approach to homozygosity.

The food used in the experiments was the customary banana-agar jell. The bananas used throughout the experiments were uniformly overripe (the skins dark brown), but not decayed, and a 1 percent agar-agar me-

dium was used. The food was poured to a depth of 25 mm. in 1×4 inch vials and yeasted 24 hours before the parents entered the vials. The same batch of food was used at both temperatures and for all time intervals in any one trial.

In the final experiments the same parents produced the eggs for the trials at 30° and 32°, 31° and 32°, and 31° and 33° C.; repeated trials being alternated between these temperature combinations. Thus comparable data should be produced for these four temperatures. One-hour egg-laying periods at 25° with eight pairs of flies per vial were used for the experiments at 30°, 31°, and 32°. The number of eggs deposited in each vial was below the limits of "the crowding effect" on the wings. These vials remained in the 25° incubator for 24 hours. At the end of this period practically all the eggs had hatched HARNLY (1929). Preliminary tests had shown no temperature effect on the wings during the first 48 hours of development. The vials were then placed at two of the experimental temperatures. Eggs from the same parents were placed at all time intervals at both temperatures, thus distributing any residual genetic variability uniformly throughout the experiment. Beginning at 48 hours of total development and every 4 hours thereafter sets of vials were returned to the 25° incubators for the completion of development and the emergence of the adult flies.

A temperature of 33° was nearly 2° above the lethal point for the complete development and emergence of the flies of this vestigial stock. A procedure was developed which would carry a fair number to puparium formation at 33°. A 3-hour egg-laying interval was used and the first 48 hours of development took place at 25°. The larvae in vials transferred at this time from 25° to 33° entered the temperature-effective period some twelve or more hours later. Beginning at 72 hours of total development and every 12 hours thereafter sets of vials were transferred from 33° to 25° for the completion of development and emergence of the adult flies. The larvae had great difficulty in forming puparia at 33°. Most of them moved up on the walls of the vials and crawled around on the surface of the glass for several hours until death resulted apparently from excessive drying. To overcome this hazard the larvae were removed from the walls of the vials with a moistened camel's hair brush every 2 to 4 hours and returned to the surface of the food during the puparium formation period at 33°. With this procedure many of them succeeded in forming puparia and, if placed at a lower temperature presumably before the time of pupation proper, succeeded in completing their development and emerging as adult flies. No emerged flies have been obtained from total development at either 32° or 33° with this stock. Total and partial development was tried repeatedly at 34°, 35°, and 36° without much success.

The accuracy of the incubators used was $\pm 0.05^\circ$ C., the temperatures being determined through the double glass door with burette readers from standardized thermometers graduated to 0.1° C. The incubators used in our laboratory for temperature work are all small units with only one shelf and an electric fan maintains a forced circulation of the air within the incubator. The thermostats are grids of glass tubing containing toluol and are closed by a mercury column having a movement in the capillary arm of an inch and a half per degree centigrade. The thermostat carries a very small current thus preventing sparking and fouling of the mercury surface and it controls the polarity on the grid of a radio tube. This in turn operates a telegraph relay carrying on its rocker arm a tube in which the heating circuit is made and broken in mercury, thus removing the common difficulty of frozen contact points in the heating circuit and the resultant death of the cultures. The lag is negligible and the heating circuit is made and broken on an average of once in 60 to 90 seconds. The air accuracy of these incubators is $\pm 0.05^\circ$ C. but tests with thermocouples placed in the surface of the food in which the larvae are feeding show no perceptible fluctuation between the on and off position in the temperature of the food surrounding the larvae. This control mechanism will maintain a constant temperature for months without any adjustment. The 25° incubators were kept in a cold room (10° C.) which had a variation of less than 1° and the 30° , 31° , 32° , and 33° incubators were kept in a room where the temperature varied from 20° to 22° C.

The right wings of the emerged flies were removed under a binocular microscope, mounted in 95 percent alcohol under a cover slip, projected with a compound microscope and a 500 watt lamp, and drawn. The linear magnification was 115. The lengths were determined by projecting a Leitz 2 mm slide ruled to 0.01 mm onto the drawings. The areas were determined with a Keuffel and Esser compensating polar planimeter no. 4242. The mean values for each time interval were determined from the lengths and areas of an average of fifty males and fifty females except the last few points at each temperature. Due to the high death rate for long exposures these points are the mean value of twenty-five to forty surviving individuals. Six trials were made at each temperature.

TEMPERATURE-EFFECTIVE PERIODS

A. Preliminary tests

The preliminary tests on the temperature-effective period were reported in London (HARNLY 1930 b). They were made on the twelfth generation of brother-sister matings with transfers from 31° and 32° to 29° and reciprocal transfers from 29° to 31° C. Since the preliminary experiments had shown that the temperature-effective period began late in the third day

of development it was not necessary in the final tests to make transfers from the high temperatures to 25° before the first 48 hours of development had been completed. This allowed a check interval at the high temperatures of over 12 hours before the beginning of the critical period.

B. Periods and growth curves for length and area

The final experiments were performed at 30°, 31°, and 32° with transfers at 4-hour intervals to 25° C. and at 33° with 12-hour transfer periods. These experiments extended from the thirty-first through the fiftieth generation of the sib matings. In figures 1, 2, and 3 are plotted the mean length

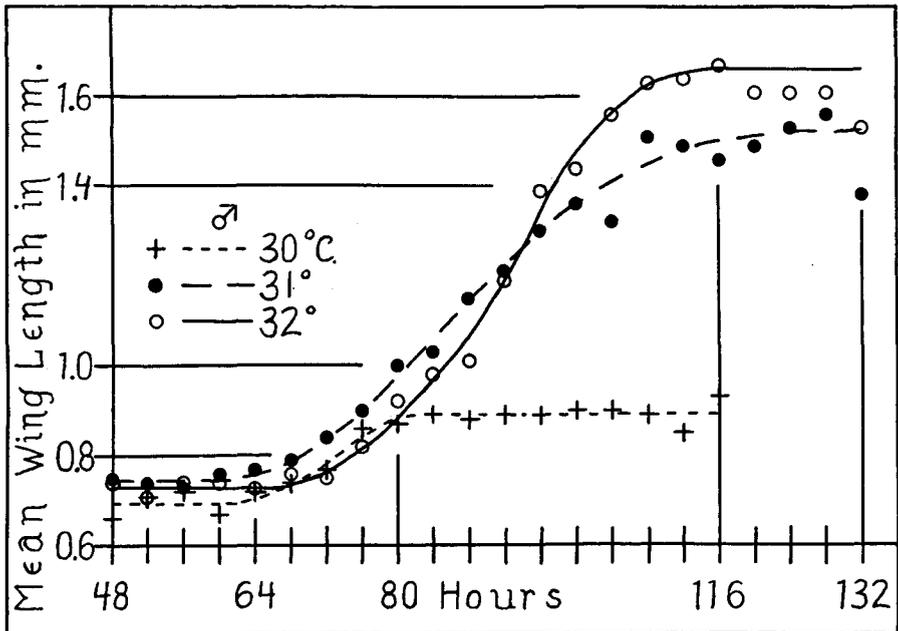


FIGURE 1

of the male and female vestigial wings for the total developmental time intervals at which the individuals were transferred from the higher temperature to the 25° incubators for the completion of development and emergence.

From the curves (smoothed by eye) it is evident that the temperature-effective period for wing length began for the males at 30° between 60 and 64 hours and ended between 80 and 84 hours of total development, a duration of some 20 hours, and terminating 27 hours before the mean time of puparium formation (111 hours). At 31° the rise began in the curve for the males at the same time as at 30° (that is, 64 hours) but instead of leveling off at 84 hours it continued upward as a sigmoid curve approaching its asymptote at 112 hours, the mean time of puparium formation for this

stock at 31°. A rise of 1° had affected neither the length of the egg-larval period nor the inception of the temperature-effective period but had changed the termination time; its duration being prolonged from 20 hours to 48 hours, an increase of 140 percent, accompanied by a 67 percent increase in wing length. Thirty-one degrees was close to the lethal point and 32° was above it for total development. Furthermore the larval period was prolonged something over 24 hours at 32° and the initiation of the temperature-effective period was retarded approximately 4 hours (68 hours) in comparison with 30° and 31° (64 hours). The duration of the effective period was again 48 hours (to 116 hours), though puparium for-

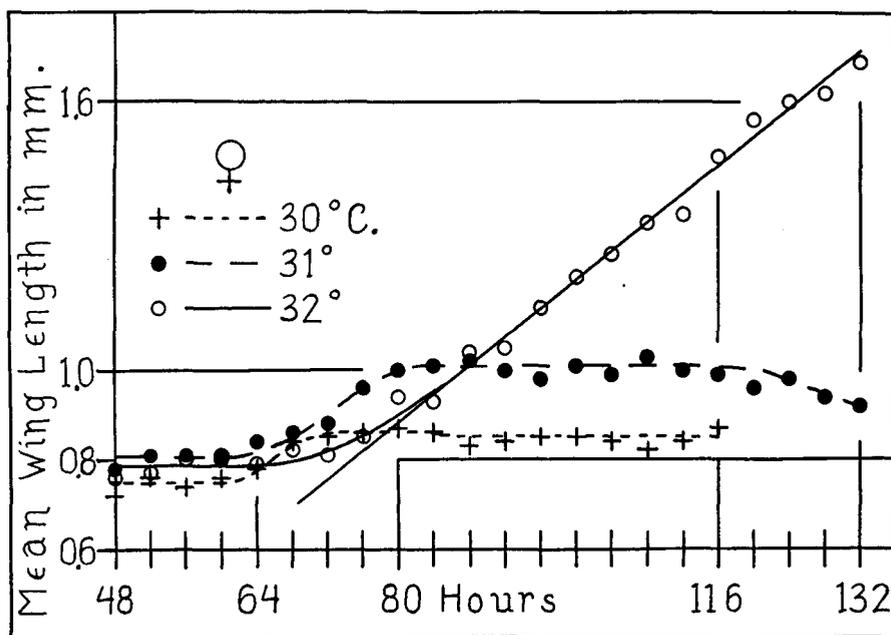


FIGURE 2

mation did not come until much later. The wings of the males at 32° were longer than those developed at 31°; this increase of 1° had not affected the duration of the temperature-effective period but did result in a higher maximum rate of wing development. The major change in the vestigial wings of the males at 32° was in the area. Due to the high mortality during the latter part of the developmental period at 33° only sufficient data was obtained to establish the five points between 72 hours and 120 hours. The linear equation

$$y = 0.01592x - 0.357$$

appears to describe these five points.

The female period for wing length at 30° began between 60 and 64 hours and ended between 68 and 72 hours of total development (puparium

formation 107 hours), an effective period of some 8 hours. Commencing at the same time at 31° the effective period was lengthened from 8 to 20 hours, the curve leveling off between 80 and 84 hours. The termination point for the females at 31° was not associated with puparium formation which came some 30 hours later (114 hours). The growth curve for the females at 32° started approximately 4 hours later than at 31° but instead of leveling off around 84 hours it proceeded upward apparently as a straight line through the rest of the time interval examined. The linear equation

$$y = 0.01592x - 0.391$$

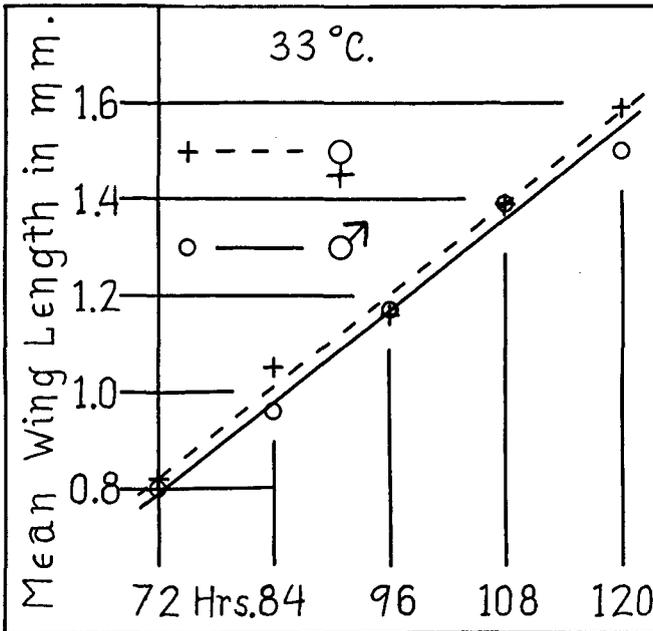


FIGURE 3

appears to describe all the points determined during this temperature-effective period with the exception of the first few points. It is obvious from the data and an inspection of the figures that the rises of 1° from 30° to 31° and from 31° to 32° though they did not significantly affect the inception of the temperature-effective period in the females did markedly affect its termination, the periods at 30°, 31°, and 32° being respectively 8, 20 and some undetermined period more than 70 hours in duration. The five points at 33° for which sufficient data was obtained appear to fit the equation

$$y = 0.01592x - 0.326.$$

The equations for the females at 32° and the males and females at 33° differ only in the intercept, the slope being the same for all three.

The close fit of the straight line drawn for the females at 32° to all the points determined experimentally, except the first few points in the temperature-effective period, raises the question as to whether or not the beginning of this period was sharply defined in time. From the curves in figures 1 and 2, either the temperature-effective period began at the same moment in time for all the individuals and the rate increased during the first few hours, or individuals all of the same chronological age entered this critical period of ontogeny at different times and each of the first two or three points represented more and more individuals in the effective period. Even the most superficial examination of the drawings for either

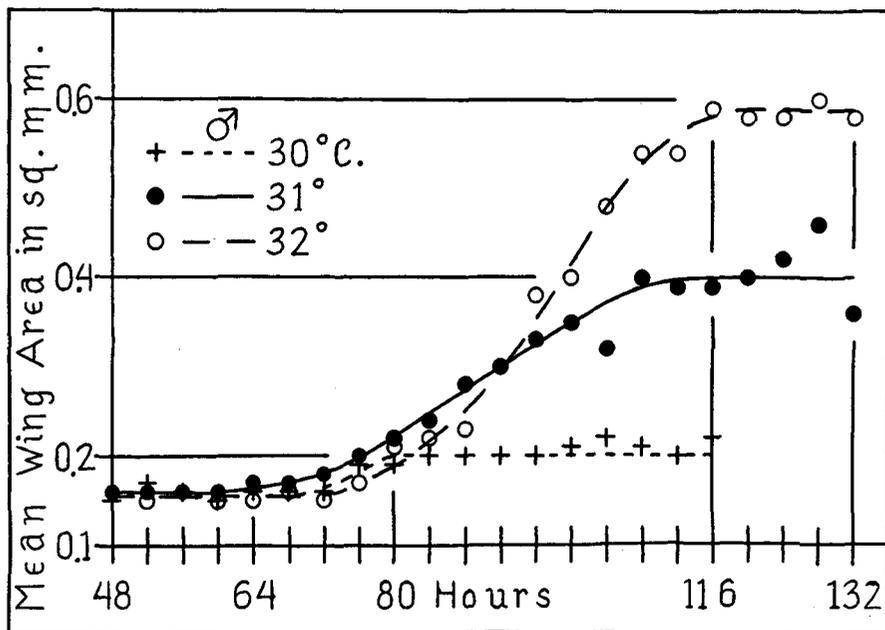


FIGURE 4

sex at any one of these temperatures demonstrates conclusively that the latter alternative was correct for both the beginning and end of the period. The temperature-effective period as used here denotes that interval in time from the entrance of the first few larvae into the critical period until the last have passed beyond it. The value is that of the whole population. In work of this type with *D. melanogaster* it is impossible to determine this value for a single individual since only one measurement in time can be made on it. The results presented here make possible a new technique by which more accurate values will probably be obtained in subsequent work with other alleles at this locus. Attention has been called to this problem in recent work on the scute and Bar loci (CHILD 1935, MARGOLIS 1935).

The wing areas are plotted against time of transfer in figures 4, 5, and 6. When one considers the data for the males at 30°, 31°, and 32° in figures 1 and 4 it is obvious that there are only minor differences in the curves and time intervals for the length and for the area of the wings. The effective period for wing area began slightly later than that for wing length. It would seem that the beginning of the period involved a slight increase in length at the expense of the width with no resultant change in area. The termination times for both length and area seem to have coincided at each temperature. For area as for length the duration of the temperature-effective period was prolonged markedly with a rise of 1° from 30°

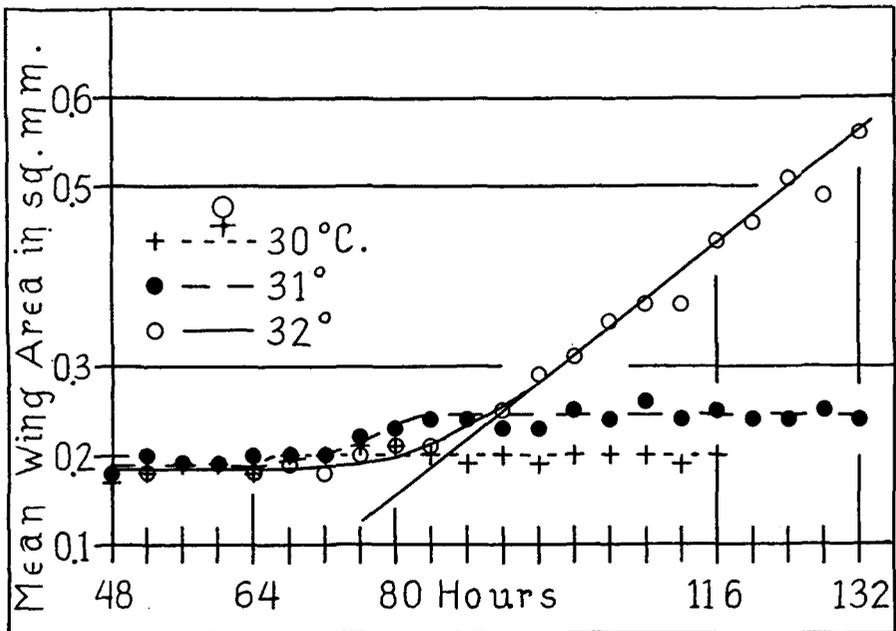


FIGURE 5

to 31° and there was a pronounced increase in the maximum rate with no significant change in the duration at 32°. The major change in wing form from 31° to 32° was in the width and area of the wing accompanied by only a minor increase in wing length. The curve for area at 33° was very definitely not linear and the wing forms were similar to those at 32° for the males.

The changes in wing length and wing area for the females were likewise very similar. The beginning of the temperature-effective period seemed to come a little later for wing area than for wing length but they both ended apparently at the same time for each temperature. The rises of 1° from 30° to 31° and from 31° to 32° each lengthened the effective period significantly. The five points determined for wing area of the females at 33° were not linear.

The sexual dimorphism previously reported at high temperatures (ROBERTS 1918; HARNLY 1930a, b; STANLEY 1928, 1931) was obviously due to differences between the males and the females both in rates of "wing formation" and durations of the effective period at each temperature. At 30° the length of the temperature-effective period of the males was several times that of the females. The effective period for the females at 31° was 20 hours (male duration at 30°) and the curve was practically identical in time with that for the males at 30°, but with a higher value throughout. However, the male duration had changed at 31° from 20 hours to 48 hours, continuing the sexual dimorphism at this temperature. A rise of 1° did not modify the inception of the temperature-effective period but had retarded its termination differently for the two sexes. The same length and area was attained in the wings of the males and the females at 32° but it is very evident from the curves that the growth processes were markedly different and the effective period was probably considerably longer for the females than for the males. If they could have been carried for a longer period at 32° the curves indicate that the wings of the females might have been larger than those of the males at this temperature. In the trials at 32° sets of vials were carried for transfer periods considerably beyond 132 hours since it was known that puparium formation came later there. Unfortunately 132 hours was practically the time toleration limit and only a few individuals were obtained beyond that point. These occasional survivors had still larger wings and, though they do not prove it, agree with the assumption that if development could go through to completion of the temperature-effective period for the females at 32°, the two sexes would still show a difference in wing size, but at this point the wings of the females would be larger than those of the males; a complete reversal of the sexual dimorphism to that found in the wild type wings.

In the light of this assumption the curves for the males and the females at 33° are of interest. The mean values for the wings of the females were larger than those of the males at the five points examined between 72 and 120 hours (figures 3, 6), a reversal of the position of the growth curves of the two sexes for vestigial. It has been shown that the wild type wing and many other structures vary inversely with temperature (LUTZ 1913, ALPATOV and PEARL 1929, ALPATOV 1930b, EIGENBRODT 1930, IMAI 1933, STANLEY 1935). STANLEY (1935) has found that the wings of homozygous long-winged (wild type) females were consistently and markedly longer than those of the males for each transfer interval during the temperature-effective periods at 17°, 27°, and 30° C. and the longer the developmental period passed at the higher temperatures, the smaller the wings. Both variation with temperature and with time and the relative sizes of the

wings of the two sexes were reversed in homozygous vestigial flies as opposed to the normal allele. But at 33°, though the wing size still increased with time its relative size for the males and females had been changed toward the wild type condition. This indicates that there had been a change in the developmental processes producing in these curves of the male and female vestigial genotypes at 33° a partial simulation of the normal long-wing genotype growth curves. However it was only a partial simulation since wing size for the vestigial flies still varied directly with the length of time the larvae were exposed to 33°. Furthermore there was never any close approach to the normal phenotype in either sex genotypi-

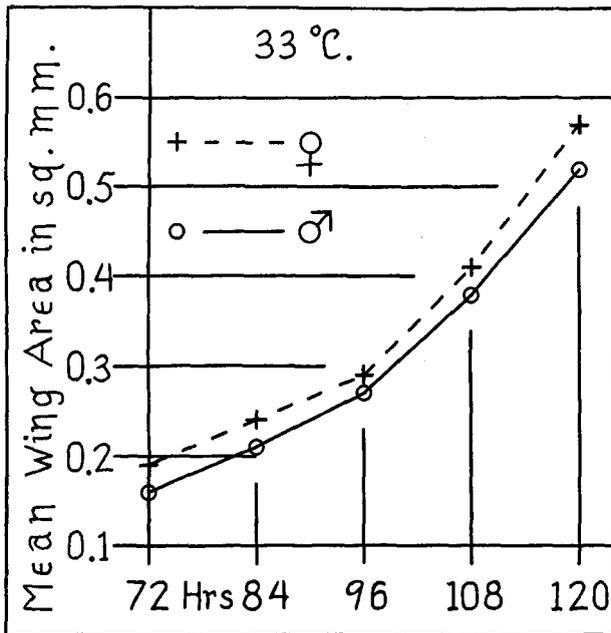


FIGURE 6

cally homozygous for vestigial. As I have pointed out before (HARNLY 1932), the mutant gene vestigial seems to determine the minimum and maximum size of the wings, the potential size being determined by the vestigial gene together with other genes affecting the wings and the exact expression is determined by the gene complex acting in a specific environment during development.

From the data presented above it was evident that the temperature-effective period ended long before the time of puparium formation for the males and the females at 30° and for the females at 31° though here the two events seemed to coincide in the males. At 32° the effective period terminated in the males long before puparium formation but this relationship could not be determined for the females due to the lethal effect of

exposures beyond 132 hours at this temperature. An experiment was performed to determine whether or not the effective period had ended in the males by the time puparia were formed at 31°.

C. Termination test

Eggs from the same parents were obtained by repeated 1-hour egg-laying periods and allowed to hatch at 25° (24 hours development). The vials were then transferred to 31° for the larval period. With the beginning of puparium formation hourly examinations were made and the newly formed puparia isolated on agar-agar slants in vials. Each hourly set of puparia (prepupae) from a given vial were divided into three groups, consequently any differences due to the number of hours passed in the egg-larval period would be uniformly distributed through the test. At each hourly isolation the first group of puparia were immediately placed at 25° for further development and emergence; the second group of isolated puparia passed an additional 6 hours at 31° and were then transferred to the 25° incubators; and the third group spent 12 hours more at 31° (by which time pupation proper had occurred in all of them) before they were placed in the 25° incubator for further development and emergence. There was no further increase in size due to exposure to 31° beyond the hour of puparium formation.

TEMPERATURE AND GENERAL RATE OF DEVELOPMENT

Any interpretation of the results depends on whether these high temperatures affected proportionately or disproportionately the duration of the effective period and the duration of the general developmental periods, especially the duration of the egg-larval period. The following experiments were performed to answer this question.

A. Methods

The usual procedure for the exact timing of development was followed (LI 1927, HARNLY 1929). Single pair matings of the inbred vestigial flies were used. The eggs were deposited on blotting-paper slips which were then placed on end in 1×4 inch vials containing food 25 mm deep. All the eggs were laid during 1-hour periods at 25° C. At the end of this interval the slips were removed and so distributed that approximately equal numbers of eggs from each female were placed at 25°, 29°, 30°, 31°, and 32°. All subsequent development took place at the temperatures indicated. Hourly examinations were made during the hatching period and at the time of puparium formation. Each puparium was placed on a 2 percent agar-agar slant in a separate vial. The time of emergence and sex of each adult was determined by hourly examinations of these isolated puparia during the emergence period.

B. Effects on the rate of development

The mean duration of the embryonic period (hours required to hatch the eggs) was 21.7 hours at 25°. This was practically the same value found previously for vestigial flies and the frequency curve conformed to the earlier one showing no signs of a bimodal distribution (HARNLY 1929). The frequency distribution curves for hatching at the higher temperatures were also simple sharp unimodal curves. The maximum effect occurred between 25° and 29°, and over the range of 29° to 32° there was no significant change in the duration of this developmental period (19.4 to 19 hours). The major effect of temperature on the length of the embryonic period occurred below the critical temperatures which were found to increase the size of the vestigial wings (30° ♂♂, 31° ♀♀).

The durations of the egg-larval periods (hours from egg-laying to puparium formation) for the males and the females are stated in table 1. An increase of 4° (25° to 29°) reduced the egg-larval period 17 hours (14 percent) for the males and 10 hours (8.6 percent) for the females, these changes appearing in a temperature range below the critical points for the wings of the males and the females. A rise from 29° through the critical point for the vestigial wings of the males to 30° produced an increase of 6.7 hours in the egg-larval period of the males, but no change occurred in the duration of the egg-larval period for the females. Conversely a rise in temperature through the critical point for the wings of the females from 30° to 31° had only a very slight effect on the egg-larval period of the males but lengthened this period 7.2 hours for the females. The lethal point for complete development of the vestigial winged flies was practically reached at 31°, very few adults emerging, and had been passed at 32°. Consequently the egg-larval period was determined for those larvae which formed puparia at 32° and as no adult flies emerged the sexes were not separated.

TABLE 1
Duration of developmental periods in hours, 4 trials.

° C.	EGG-LARVAL		EGG-LARVAL-PUPAL	
	MEAN ± P.E.	σ	MEAN ± P.E.	σ
25♂	121.35 ± 1.37	10.37	229.81 ± 1.48	11.18
♀	117.37 ± 1.09	8.38	219.42 ± 1.39	10.50
29♂	104.14 ± 0.82	6.53	192.69 ± 0.91	7.26
♀	106.78 ± 0.87	8.26	192.63 ± 0.96	9.13
30♂	110.86 ± 1.61	12.83	195.79 ± 1.10	8.75
♀	106.83 ± 0.82	5.95	189.88 ± 0.83	6.02
31♂	112.22 ± 1.03	4.59	199.78 ± 1.42	6.32
♀	114.00 ± 0.97	3.22	197.80 ± 1.63	5.42
32	140.07 ± 1.64	18.55		

From the egg-larval-pupal periods and the differences between them and the egg-larval periods in table 1 there was evidently little change in this period above 29° through the critical temperatures for the males and the females (30° and 31°). The pupal period may be ignored in this discussion since there was no differential effect of temperature through the critical range, and since it has been shown above that the temperature-effective period fell entirely within the larval period of development.

The difference of 6.7 hours in the egg-larval periods between the males at 29° and 30° represented an increase of 6.4 percent in this period and was 3.72 times the probable error which is just statistically significant and is based on only a very limited number of surviving individuals. In comparison with this small and questionable increase of the egg-larval period from 29° to 30° a pronounced lengthening of the wings by some 35 percent occurred with the same rise of 1° in temperature (HARNLY 1930a). Furthermore at 30° the temperature-effective period was 20 hours and bore no obvious relation to either the small questionable increase of the egg-larval period or to the periods found by STANLEY (1931) at 17° and 27°. As has been shown above with identical egg-larval periods at 30° and 31° there was an increase of 140 percent in the duration of the temperature-effective period and the wings were lengthened 67 percent at 31°. The same situation was found throughout for the females. In the light of these facts and all the data it seems probable that the effects of temperature above 29° on the duration of the major periods of development (embryonic, egg-larval, and egg-larval-pupal) offer no explanation of either the critical temperatures or the sexual dimorphism. Temperature through its effect on the duration of the critical period for wing development and the rate during that period affects not only the length and area of the wings produced, but also through the same processes the wing form or pattern. The result is not simply more wing of the same kind but a definite ordered progression toward the normal type of wing.

GROWTH PATTERN OF WING FORM

Genotypically homozygous vestigial flies show an interesting succession of phenotypes when reared at 29°, 30°, 31°, and 32°. Typical vestigial wings are produced at 29° and all temperatures below this point. The wings of the females reared at 30° are still typical but those of their brothers are much larger and in appearance markedly over-sized vestigial wings. The wings of the females at 31° have increased greatly in size as the male wings did at 30° and in form are the same type of over-sized or giant vestigial wing. But the wings typical for their brothers at 31° are no longer vestigial but the equivalents of the mutants strap to antlered in phenotype. Finally at 32° these genotypically vestigial males and

females have wings that are phenotypically the equivalent of exaggerated notched, or the heterozygote Wild/Carved mutants with parts of the lateral margins missing in many cases. The changes of this genotype with temperature simulating phenotypically other alleles of vestigial reared at 25°, together with the complete progression of the genotype dimorphos vestigial through the vestigial allelic series from the phenotype vestigial at 16° to strap, antlered, Snipped, notched, Carved, vestigial-Beaded, nick, nicked, and finally wild or normal at 32°, and the fact that the homozygous genotype dimorphos vestigial through the temperature range of 16° to 32° duplicated all of the phenotypes produced in MOHR'S (1932) combinations of mutants at the vestigial locus at 25° C., led to the propounding of a theory of wing pattern in development in time and the

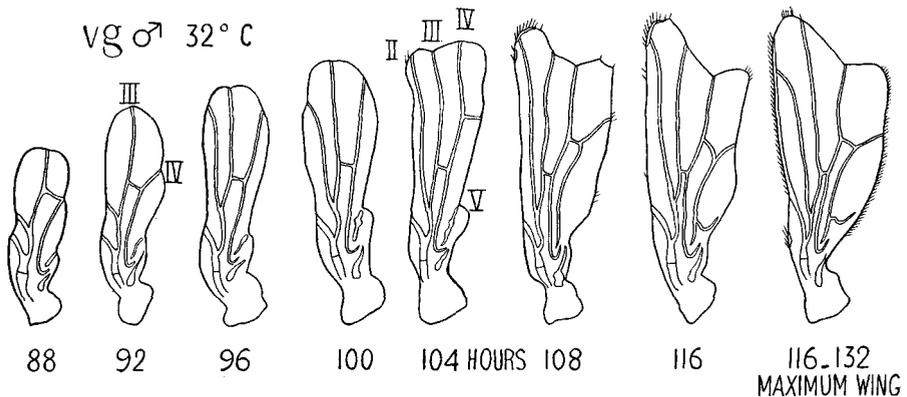


FIGURE 7

hypothesis that mutations at the vestigial locus involved changes in the rate and duration in time of wing development (HARNLY and HARNLY 1935). It must be remembered that mutations at this locus show changes in the form and amount of parts of the wing present, not changes in size only as in some of the other wing loci. The pattern theory advanced involved first growth longitudinally in the region of the II, III, and IV veins with no marked development in width beyond them, followed by growth mainly in the regions of the I and V veins, resulting in a pronounced increase in the width and area of the wing, the last part of the wing developed being the distal margin in the region of the III and IV veins. The same phenotype series has been found for the vestigial genotype in time of development.

This pattern series in ontogeny is shown in figure 7. These wings were the mean types for males spending the number of hours indicated at 32° and completing their development at 25°. The wing at 88 hours was simply an enlarged vestigial wing with a length of 1.01 mm. It was equivalent to

the wings of the homozygous vestigial males reared at 30° (HARNLY 1930a). Growth from then on was mainly longitudinal in the region of the II, III, and IV veins. Twelve hours later (100 hours) the wing was passing through a stage resembling the vestigial-strap mutant, many of the wings being indistinguishable from the published figures of strap wing. Four hours later (104 hours) the wings were similar to the allele antlered wing figured by MOHR (1932). Beyond this interval the bulk of the growth involved the regions of the I and especially the V veins with only minor increases in length. The maximum wing type was obviously a slight exaggeration of the vestigial allele notched-wing. The vestigial genotype during its development at 32° had passed in phenotype successively through the alleles vestigial, strap, antlered, and notched. The sequence of wing patterns for the females at 32° was the same, but had different time values since the rate was much slower in the females (see figures 1 and 2, 4 and 5). There was a definite pattern of wing development in time at 32°.

The same series can be demonstrated at 31°. The duration for the males at 31° and 32° was 48 hours but the rate at 31° was much slower. Consequently the mean wing attained at the end of the critical period was between the two wings shown for males at 32° of 100 and 104 hours of development. The period for the females at 31° was much shorter than that at 32° and the wings only reached a value between those of 88 and 92 hours for the males at 32° (figure 7). Changes in duration and rate with temperature changes had not affected the fundamental pattern of wing development; they simply determined the extent of the expression of that pattern in space. These changes in wing form with changes in duration or rate, together with the sequence of wing forms in time shown in figure 7, are in complete agreement with our hypotheses stated above and advanced recently (1935).

DISCUSSION

The facts established by the data presented here are: (1) beginning 1° below the critical temperature and extending through 29°, 30°, and 31° there were no significant changes in the duration of the embryonic, egg-larval or egg-larval-pupal periods; (2) their durations were practically identical for both sexes and all three temperatures; (3) the beginning of the critical period affecting wing formation at 30°, 31°, and 32° for the males and the females was the same (approximately 64 hours of total development); (4) the termination of this critical period varied with the sex and the temperature, its duration for the males being 20 hours at 30° and 48 hours at 31° and 32°, and for the females 8 hours at 30°, 20 hours at 31° and some undetermined time greater than 70 hours at 32°; (5) the rate of the developmental reactions involving the wings changed with temperature, that

is, the duration for the males at 31° and 32° was 48 hours but the maximum rate at 32° was higher than that at 31° resulting in a much larger wing at 32°. With a constant developmental period for the whole organism, and a constant point in that period for the inception of the reactions involving the development of the wings, there were changes in both the duration and the rate of these reactions with changes in temperature. Obviously we are dealing here with an example of a differential effect of temperature on various, as yet undetermined, developmental processes. (6) From the wing types produced by homozygous vestigial, homozygous dimorphos vestigial, homozygous pennant, heterozygous pennant/vestigial, and dimorphos/dimorphos pennant/vestigial through the range of 16° to 32° and from the wings produced in the temperature transfers just discussed it has become evident that (a) there is a definite pattern of wing development in time, (b) the degree of expression is dependent on the duration and rate of processes occurring in the larval period of the vestigial genotype, and (c) apparently mutations at the vestigial locus affect the duration and rate of these developmental processes. Any attempted general interpretation of these facts must fit both the data presented here and the results of STANLEY (1931, 1935).

STANLEY (1931) reported on the temperature-effective periods at 17° and 27° C. for vestigial wing and recently (1935) on those at 30° and 31° for vestigial wing and 17°, 27°, and 30° for the wild type normal allele of vestigial wing together with the heterozygotes at 17° and 27° C. With two temperatures common to his work and the data presented here certain comparisons can be made and conclusions may be drawn. Both his work (1931) and mine (1930a) show the same critical temperatures for the vestigial wings of the males and for the wings of the females. But there are significant differences for wing length at the higher temperatures resulting in somewhat different curves; differences on the whole constant and consistent in all our experiments.

His wing length for total development of males at 29° was 0.98 mm and mine 0.74 mm; at 30° we obtained 1.52 mm and 1.00 mm respectively; and at 31° we differed by 1.64 mm and 1.70 mm. The same differences were again consistently present in the maximum mean wing length for the males in his transfer experiments from 30° and 31° to a lower temperature and in the data presented here for the transferred males at those temperatures. Similar consistent differences throughout were evident for the females. My own stock was inbred and selected, STANLEY'S stock was inbred but unselected. It is obvious, I believe, that we were dealing with different sets of sex-linked and autosomal genes modifying the action of the vestigial gene. The validity of such an assumption is indicated by STANLEY'S selection and tests at the end of his experiments (1935). He

found that some of his heterozygous Wild/vestigial flies had "beaded" wings. From his original inbred and unselected vestigial $\bar{S} V A$ line he selected two new lines, $S V B$ comparatively free of the modifying genes, and $S V C$ practically homozygous for them. These three vestigial stocks were then tested at 29.5° and found to be significantly different in wing length. He says "Since 29.5° is very close to the 'critical temperature' for wing length, two assumptions are possible: (1) the critical temperature is shifted upward by the presence of the factors, or, (2) the factors have a direct inhibitory effect upon wing length. It is impossible, with the data available, to say which of these two assumptions is nearer the truth." In his case either may be correct. We have shown recently (HARNLY and HARNLY 1935) that the critical temperature of vestigial may be lowered as much as 5° by the presence of the sex-linked modifier *dimorphos*. From the consistency of the values obtained throughout his work and throughout mine and the evidence advanced for the presence of modifying genes we may assume that those points in which our results differ were due to the presence of different sets of modifying genes.

In view of those differences it is significant that we are in practical agreement on the moment in development at which temperature becomes operative. We have both found the first indications of this action in the vestigial flies (males and females) around 64–68 hours of development at 30° and 31° and I have found the same point at 32° . STANLEY reports 68 hours as the critical point at 30° for homozygous Long-winged flies. The similar time of inception of the critical period in development for the wings at these temperatures in the homozygous normal allele of vestigial and in homozygous vestigial with either of two sets of modifying factors would all indicate that its initiation was probably not due so much to the vestigial gene or its normal allele as to the reaching of some general stage or process in larval development. From CHEN's work (1929) this stage morphologically cannot be either the first appearance of the dorsal mesothoracic buds, which comes much earlier in development, nor the first appearance of the wing buds which comes much later in ontogeny. The work of ALPATOV (1929, 1930a) on the development of wild and vestigial larvae indicates that the most probable morphological point of attack is the molt between the second and third instars. Tentatively then I am assuming that the critical period in wing development at these high temperatures is initiated in time by the molt from the second to the third instar. This assumption has one major advantage over the others that might be advanced, the facility with which it may be proved or disproved. By the use of a new technique it is hoped to substantiate or invalidate this assumption in the determination of the temperature-effective period for pennant, a new regressive mutation from vestigial to a recessive wild or normal type wing.

Our close agreement on the beginning of the temperature-effective period together with both the marked differences in our end points in time at 30° and 31°, and the pronounced changes I have found in the time of termination at 30°, 31°, and 32°, indicate that the beginning and end of this period were determined by different and independent processes. Furthermore, as I have shown above, the processes ending this critical period were not associated with the effects of temperature on the general rate of development. Without any change in either the length of the egg-larval period or the time at which the temperature-effective period began at 30° and 31°, there was an increase of 140 percent in the duration of the critical period with this rise of 1° for the males of our vestigial stock. Similar results were obtained for the females. This rise of 1° in some way enabled the vestigial gene to continue its activities much longer in association with the rest of the gene complex controlling those processes leading to wing development. This interpretation agrees with the assumption that the vestigial mutation produced a gene operating within the refractory temperature limits for a much shorter interval of time and a different rate from its normal allele, consequently producing during ontogeny less of the precursor of the ultimately formed wing bud. The differences in the termination points and the duration of the egg-larval periods as related to the duration of the temperature-effective periods determined at 30° and 31° by STANLEY and myself were probably due to the different sets of modifiers present in our vestigial stocks. That even a single modifier may have a profound effect on the action of the vestigial gene was shown by the fact that the mutant sex-linked modifier *dimorphos* enabled the vestigial gene to produce wild-type wings at these high temperatures (HARNLY and HARNLY 1935). We may conclude that the initiation of the temperature-effective period at these high temperatures was dependent on the attainment of some developmental stage (probably the molt from the second to the third instar) and that its termination was independently determined by the response of the wing gene complex to specific temperatures, the consequent durations in my stock being disproportional to the duration of the larval periods in which they occurred. The rates of the processes concerned with wing formation were likewise dependent on or related to the temperature experienced during the critical period of development. Any attempt to explain further the action of the vestigial gene and its relation to temperature would be hazardous at this time. The responses of its allele *pennant* and the heterozygote *pennant/vestigial* in total development through the temperature range of 16° to 32° preclude any explanation in terms of the data available at present. These experiments with *pennant* and *pennant/vestigial* have been completed and will be reported shortly.

GOLDSCHMIDT in the course of his extensive studies on *Lymantria dispar* presented in 1920 a general theory of the action of the genes in development, a theory which he has evolved more completely since then (GOLDSCHMIDT 1934, complete bibliography). This theory is based on his work on intersexuality and pigmentation patterns in the larvae and assumes that the genes affect the rates and durations of processes in ontogeny. The data presented here on vestigial are in agreement with his hypothesis.

SUMMARY

1. The temperature-effective periods for the length and area of the vestigial wings of an inbred selected stock of *D. melanogaster* are reported for 30°, 31°, and 32°.

2. The critical period began at approximately 64 hours for both sexes at these temperatures. It had a duration of 20 hours at 30° and 48 hours at 31° and 32° in the males and 8, 20, and some interval more than 70 hours for the females. Rises of 1° at these temperatures did not affect the inception of the period but markedly affected its termination and the rate; that is, in the males at 31° and 32° with no change in duration there was a marked increase in the rate.

3. The differences between the sexes in duration and rate with increases of 1° afford a formal explanation of the sexual dimorphism of the wings.

4. A complete reversal of the relative sizes of the wings of the males and the females was found at 33° for all developmental points examined.

5. There was never any close approach to the wild type wing at these high temperatures. The growth pattern of wing form in time is shown and related to the alleles at the vestigial locus and the theory of wing pattern and mutation stated in an earlier paper.

6. The duration of the embryonic, egg-larval, and egg-larval-pupal periods were determined at 25°, 29°, 30°, 31°, and 32°. Pronounced differences in the length of these periods were found between 25° and 29°. No significant changes occurred in their duration at 30° and 31°. The effects of temperature above 29° on the duration of the major periods of development offer no explanation of the critical temperatures, the sexual dimorphism, or the increase in wing size. The results must be due to a differential effect of temperature on various processes during ontogeny.

7. The inception of the critical period in development is interpreted as occurring at the molt from the second to the third instar at these high temperatures; its termination is apparently independently determined by the gene complex (including sex) and the temperature experienced, the consequent durations being disproportional to the durations of the larval periods. The rate is likewise dependent on the gene complex and the temperature.

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