TEMPERATURE EFFECTS ON SCUTE IN DROSOPHILA MELANOGASTER

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Introduction

During the past twenty-five years a considerable amount of literature has appeared in biological journals dealing with the effects of temperature upon the expression of Mendelian characters in Drosophila melanogaster. It has been generally noted that the expression of many, if not of most, mutations is conditioned to some extent by the temperature at which the fly develops. It has been shown further in each of a number of instances that the temperature effect on the adult character is produced during only part of the total developmental period, the position and length of this part depending upon the mutation concerned. This part of the period has come to be known as the "temperature-effective period" (TEP) of the character.

Studies on TEP's have been undertaken largely with the hope of unveiling the mechanism of gene action in development. Although they have not realized this hope extensively, these studies have brought to light many important observational facts, as well as some interesting speculations concerned with their interpretation.

Practically all of the studies thus far reported have been made upon the so-called "viable range" of temperatures, by which is meant the temperatures at which D. melanogaster can develop successfully from egg to adult. The

study reported here, suggested by Professor Sturtevant, was made at temperatures above this range, using a mutation whose responses to viable temperatures were at that time in the process of being determined. The results prove to be of interest both by themselves and in contrast with the results of the subsequently published lower temperature studies.

I propose first to review the TEP literature, and then to present and discuss my own experimental data.

SECTION I.

A REVIEW OF THE LITERATURE ON TEMPERATURE-EFFECTIVE PERIODS IN DROSOPHILA

A. Introduction

No general review of the literature from TEP studies on Drosophila mutants has been published. Accordingly, this review is entirely from the original papers. The mutant characters are discussed in approximately the chronological order in which their respective TEP's were first studied.

It will be noted that the bulk of the literature is from studies on Bar eye and its alleles, and on vestigial wing and its alleles. Most of the work on the Bar series, and much of that on the vestigial series, has been done under the supervision of Professor Charles Zeleny of the University of Illinois (Urbana) or of Professor Charles Plunkett, Washington Square College, New York University (New York City). The studies on eyeless and on scute bristles were also done under the supervision of Professor Plunkett.

B. Reduplicated Legs

The first report of temperature effect on the expression of a Mendelian character in Drosophila was that of Miss Hoge (1915) on the sex-linked recessive, "reduplicated legs". She found that cultures of a pure line of this mutant manifested up to 100% of the character when maintained at a temperature of 10 to 12°C. well into the pupal period, as compared to a 5 to 10% manifestation in cultures raised entirely at room temperatures (about 22°C.).

Miss Hoge made two series of experiments to show

when the temperature effect was produced in development. In the first series, cultures began development at room temperature and were transferred at intervals to the low temperature. The results indicated that the low temperature effects were strongest when the transfers were made in the egg stage, and were progressively weaker as the time of transfer was delayed. When development had proceeded for more than four days at room temperature, there was no apparent effect of subsequent low temperature on the manifestation of the character. This indicated that the low temperature was effective only during the egg-larval period and not in the pupal stage.

In the second series of experiments, the cultures began development at the low temperature and were transferred at intervals to room temperature. From this series, it was learned that the effect of low temperature did not manifest itself until the first five or more days of development had elapsed. The effect of the low temperature increased the longer the transfer to room temperature was delayed. The effect was greatestwhen cultures were kept at the low temperature until the appearance of the pupae.

In both of these series, the temperature effect seemed to be directly proportional to the amount of egg-larval development which took place at the low temperature; and the TEP seemed to occupy the egg-larval period of development.

Miss Auerbach (1936) showed in her studies on Drosophila imaginal discs that certainly from the early

larval period the limb buds are in visible process of growth. She suggested that the effect of "reduplicated legs" is strongest on the embryonic processes leading to the formation of these buds, since the most notable effect of the low temperature manifested itself during the embryonic period. The TEP, however, was not closely enough determined to make profitable an exact analysis from Miss Hoge's data.

C. Bar Eye and Its Alleles.

1. Introduction.

Seyster (1919) was the first to report an effect of temperature upon the appearance of the dominant, sex-linked, eye-reducing gene, Bar (B). He showed that facet number in B eye is inversely related to the developmental temperature. As the temperature was lowered through the viable range from 29 to 15°C. the facet number increased, more rapidly in females than in males. Males, however, appeared to have more facets than the females at all temperatures. He found a van't Hoff Q_{10} value of 2.6 in males and 3.5 in females. From this he concludes that B acts as a chemical inhibitor of facet formation, and that its effect is stronger at high temperatures than at low. His studies on the TEP indicated it to be contained within the larval developmental period.

Since Seyster's report, many temperature investigations have been made on B and its alleles. The reports have come from Krafka (1920), Hersh (1924 - 1934), E. C. Driver (1926,1931), Luce (1926, 1931), Olive Driver (1931),

Margolis (1935 - 1936), and Margolis and Robertson (1937).

In general these studies have been made in the viable range of temperatures, from 15 to 31°C., and have been made on several B stocks as well as several alleles. These alleles include "ultra-Bar" (B^u) which manifests a more extreme effect than B, and "infra-Bar" (B^i) which produces a less extreme effect than B, as well as the wild-type eye. The studies also include work on the several heterozygotic combinations of these genes.

2. Temperature response.

B and B^u, and their heterozygotes with each other and with wild-type, show and inverse response to temperature changes. B^i shows a direct response to temperature changes, the facet number being higher at high temperatures than at low. While the B/B^i and B^u/B^i flies show facet numbers intermediate to the two alleles in each case, the temperature response is inverse, as in B and B^u.

3. Sexual dimorphism.

In all of the alleles and their combinations there is a clear sexual dimorphism in facet number, the males consistently having a few more facets than the females at all temperatures. There does not appear to be any notable dimorphism in respect to their temperature responses.

4. Position of the TEP in development.

TEP's were determined for B and its alleles by transferring cultures from one temperature to another at graded intervals in development. There appeared to be some

variation in TEP from stock to stock of B. Among the alleles there was a much wider variation. In all cases, the TEP seemed to occur within the first half of the third instar period throughout the viable temperature range.

The data of E. C. Driver, Luce, and Margolis are the most instructive concerning the position of the TEP at different temperatures. Driver found that in B the beginning of the TEP is probably constant throughout the viable temperature range, and that it probably coincides with the beginning of the third instar. The end of the TEP changes at some temperature between 20 and 22°C., and changes the duration of the TEP. Up to 20°C., the TEP covers 25% of larval life; from 22 to 30°C. it covers only 16%. He found that in B^u, on the other hand, the duration of the TEP is consistently the same at all temperatures, and consists of approximately 17% of egg-larval development. The position of the TEP, however, lies progressively later in the first half of the third instar as the temperature increases.

Luce showed that in B^i there is a slight shift in the beginning point of the TEP which gradually shortens its duration from 31% of egg-larval life at 17°C. to 24% at 27°C. The heterozygote, B/B^i, shows to a lesser extent both the effect of B^i on the beginning point and the effect of B on the end-point of the TEP. The duration of the TEP in B/B^i decreases from 24% of egg-larval life at 17° to 13% at 27°C.

Margolis worked out a method for calculating the TEP of the individual larvae from that of the entire larval population. He found, as might be expected, that the TEP of the individual is much less than that of thepopulation.

The population is made up of individuals which vary in the degree of their respective developments at a given time. The TEP of the population is the sum of the TEP's of the constituent individuals, and its length is determined by the oldest and youngest larvae present. Margolis found that in his B stock the duration of the individual was considerably less than half that of the population TEP.

From the reports of the investigators as a group it is apparent that the position and duration of the TEP vary slightly, probably because of different sets of modifying genes in the different stocks and because of differences in technique and in environmental conditions.

5. Rate of change in facet number.

The rate of change in facet number per degree change in temperature varies between alleles and to some extent between stocks of the same allele in the B series. It is generally not greatly different between the sexes of a given stock. Driver found in B a 10% increase in facet number per degree drop in temperature, between 30 and 22°C. Between 22 and 20°C. the rate changed, paralleling the change in position and duration of the TEP. From 20 to 15°C. the rate of increase in facet number was 7% per degree.

Driver's data on B^u indicate a fairly constant rate of increase in facet number throughout the temperature range. The rate was between 4 and 5% per degree drop in temperature.

In 1926 Luce reported in his B^i studies that the rate of increase in facet number per degree increase in temperature, over the entire viable temperature range, was 8.3% in

males and 7.3% in females. In his 1931 data these rates had dropped to 7.2% and 6.6%, respectively. A comparison of the data of Seyster, Krafka, Margolis, and others shows similar small changes in rate between stocks of B and alleles, at different points in time, and under different conditions.

Hersh (1927) reported that in reciprocal crosses of B and B^u, while the facet number was nearly exactly intermediate between the alleles in both crosses, the rate of decrease in facet number followed the maternal allele in each case. Further studies (1930) showed that this was true only at lower temperatures; that the rates approach each other at higher temperatures; and that at 28°C. the rates in reciprocal crosses are practically the same. In B/B^i Luce found that the rate was only slightly lower than in B.

6. Relation of facet number and temperature.

There does not seem to be any general agreement among the investigators of B and alleles as to the nature of the relation of change in facet number to change in temperature. Between 17 and 27°C. the relation may be plotted either as linear or logarithmic. While the behavior at extreme temperatures seems to favor an exponential viewpoint, it has been pointed out by those favoring a linear relation that irregularities in the linear curves at the extremes can be accounted for on the assumption that they represent irregularities consequent to the increasing developmental disturbances of the extreme temperatures.

In the case of B, the change in rate between 20 and 22°C. is such that two straight lines give a better fit

to the data, whether the calculation be on a linear or exponential basis.

7. Nature of the Bar reaction.

Following Seyster, Krafka (1920) calculated van't Hoff Q_{10} values for facet number change in the temperature range 15 to 31°C. In B^u, Q_{10} varied from 1.60 to 2.86 in this range. In B, it varied from 1.77 to 4.43. In both cases it was low in the middle temperatures and high at the extremes. Krafka concluded that, on the whole, his data supported Seyster's hypothesis that the B reaction represents a unimolecular chemical reaction.

E. C. Driver (1931) and Hersh (1934) have carried their calculations further, using the Arrhenius equation. Instead of giving a straight line as demanded by the Seyster-Krafka hypothesis, the plotted u values give a smooth curve throughout the temperature range. Driver, following Crozier (1924), interprets this to mean that the B reaction is not a "master reaction"; that we are concerned, instead, with an equilibrium between two systems. One of these systems involves the reactions of B and its alleles; the other, the reactions of the rest of the gene complex as a whole. The differential temperature coefficients of these two systems, he believes, account for the change in facet number with change in temperature. The B and B^u systems can be considered as having higher temperature coefficients than the system involving the rest of the genes concerned with development. Consequently, their restraining influence on facet formation

is greater at higher temperatures. B influences both the rate of facet formation and the duration and position of the TEP; B^u influences the rate of facet formation and to a less extent the position of the TEP. The B^i system can be considered as having a lower temperature coefficient than the system of development as a whole, and consequently affects the facet number less at higher temperatures. The effect of B^i is largely on the rate of facet formation, less on the position and duration of the TEP.

Margolis (1935 - 1936) reported studies on the temperature response of B in vestigial (vg) winged flies. He found the B vg has fewer facets at nearly all temperatures than the B stock from which it was derived; and that B vg also has a slightly longer TEP than the B stock. Since vg is known to increase the lenght of larval life, he argued that the effect of vg on B facet number can be formally explained as an increasing of the time in which the B gene can affect facet number.

Krafka (1920) was unable to detedt any effect of temperature on facet number in the wild-type eye. Rosalie Hersh (1924) reported a small steady decrease in facet number with increasing temperature, equivalent to a 2.5% decrease per degree of increase in temperature. She did not attempt to locate the TEP. Margolis and Robertson (1937) found that the effect of temperature was not constant at different points in the developmental period. There seemed to be a general increase in facet number during the embryonic stage, and a general decrease during nearly all of

larval life, the change in no case being large. In both egg and larval periods, however, there were changes in direction in the facet-temperature curve, and they concluded from the nature of these changes that there are at least four reactions responding at these different times to temperature changes.

From the results of the studies with B vg and with wild-type eye, Margolis extends the above theories of the nature of the Bar alleles and of the mechanics of their effects on development. He postulates three reaction systems which lead ultimately to the formation of ommatidia and facets. The first of these systems forms A, which determines when the region containing facet-forming material shall differentiate into ommatidia. The second system forms F, the facet-forming material. The third forms D, which catalyses the destruction of F by a chemical reaction which he denotes as

$$F + D \longrightarrow x$$

and which figures prominently in Bar activity. This third reaction system is of relatively negligible importance in the wild genotype and is in fact part of the second system. In B and its alleles it has become of much more importance in the determination of facet number. From the TEP data of the wild type eye the production of F seems to begin early in embryonic development. From the TEP data on B and alleles the reaction between F and D occupies the early part of the third instar. The onset of the reaction Margolis assumes to coincide with and indeed to be caused by the be-

ginning of the production of D by the third raction system. The ending of the reaction comes at the time at which A determines that the remaining F shall become ommatidia. The effects of the different alleles of B are upon the rate and duration of the reaction between F and D.

8. Correlation of TEP with visible embryological processes.

Krafka (1924) reported a brief study of larval embryology of the imaginal eye disc in Drosophila. He decided that the TEP of B coincides with the joining of the prospective eye disc with the larval brain ganglia. Chen (1929) reported a much more detailed series of studies on larval embryology. It is generally to his work that subsequent TEP investigators have referred. He located the prospective eye material in the late first instar, when it appears in the form of paired frontal sacs invaginated from the pharynx. He followed the subsequent growth, differentiation, and development of these sacs into ommatidia in early pupal life. Studying the effects of B, he was able to distinguish B and wild-type eyes in the mature larvae when the eye bud and ommatidia number are smaller in B. This is approximately a day later than the TEP. Driver (1931) concluded that the TEP occurs at the time when the frontal sac, having migrated back to the brain ganglia, differentiates into the optic and antennal buds.

D. Eyeless

The temperature response of the genotype of eyeless (ey) has been reported by Baron (1935). The ey phenotype is variable from "no eye" to nearly normal eye. In

highly selected and inbred stocks a large proportion of the population is without eyes. Baron found this proportion varied with the developmental temperature. In a stock selected for low frequency of eyes, an increase in temperature decreased the number of eyes. In the higher temperatures of the viable range practically no eyes were present. In a "standard" ey^2 stock, inbred but selected for a high frequency of reduced eyes, the situation was entirely different. Both the size of the eye and the number of eyes present increased with the temperature, excepting at the highest temperatures. Nearly all the flies raised at 27°C. had eyes. It is evident that the ey phenotype is much more dependent upon modifying genes than is B.

Using the "low-frequency" stock, Baron found that the TEP occurs roughly between $1\frac{1}{2}$ and $2\frac{1}{2}$ days of larval development at 25°C. The end, he believes, corresponds roughly with the time of appearance of the optic buds. Chen (1929) found that at this time ey larvae have only rudimentary buds.

Baron studied a combination of B and ey high-frequency stock. The facet number was consistently lower than in the B stock; but the TEP seemed to correspond entirely with that of B.

Baron has advanced an hypothesis to account for the behavior of low-frequency ey. He considers ey as responsible for the production of a variable amount of a substance, E, which reacts with the products, F, of the rest of the genotype to produce facets, f. F is produced in constant quantity at all but the extremes of viable temperatures.

He shows from his data that the relation of E to f is constant at least at medium temperatures. Up to an f value of 400 facets the relation of E and f is linear; from f values of 400 to 880 (upper limit in the normal eye), the relation is logarithmic. He interprets this to mean that E is an enzyme acting on the substrate, F. Below the 400 value of f, the relation of E to f depends, practically, on the amount of E present, since that amount is small compared to F. Above 400, the amount of E increases and F decreases to the extent that the E - f curve now becomes the product of two variables, E and F, and is consequently logarithmic.

E. Vestigial Wing and Its Alleles.

1. Introduction

Roberts (1918) first reported an effect of temperature upon the appearance of vestigial (vg) wing in D. melanogaster. He found that vg flies which developed at temperatures between 27 and 34°C. had larger wings than those which developed at room temperatures. The effect was produced during the larval development and not in the pupal stage.

More extensive reports have appeared from the work of many investigators in the 20 years since Robert's report, and have included studies on several vg alleles as well as vg itself. Papers have been published by the following authors: Stanley (1928, 1931, 1935); Harnly (1930, 1936); Hersh and Ward (1932); Riedel (1934); Harnly and Harnly

(1935, 1936); Li and Tsui (1936); Gable (1938); and Lashin (1938). Nadler (1926) has reported a study on vg in D. virilis.

2. Temperature response of vg.

It has been found to be general in D. melanogaster that vg wing increases in size with an increase in developmental temperature. The increase is at a relatively slow rate over the range, 16 to 29°C., and does not involve a marked phenotypic change. The rate of increase is rapid from 30° to 32°, which is the limit of the viable range for vg flies. It is accompanied by striking phenotypic changes in the wing. A clear sexual dimorphism exists in this upper range. The "critical" temperature at which male wings first begin to show the rapid increase in rate of change is near 30°C; the critical temperature for the female wing is near 31°C. The female wing at 30°C. is still small, like the male wing at 29°C. The female wing at 32°C. is still larger, and exceeds the size of the male wing, which does not increase in size with the change from 31 to 32°C.

From 16 to 29°C., the vg phenotype is practically "vestigial". But as the wing grows larger above the critical temperature, it passes through stages resembling all of the less extreme alleles of the vg series. (See Mohr 1932 for illustration of alleles.) At 32°C., vg wing is notched at the tip; but otherwise it is normal in appearance. In area, it is still smaller than the wild-type wing at 32°C.

Accompanying the changes in the phenotype of the vg wing are similar changes towards normal in the elevation

of the posterior scutellar bristles, and in the size and appearance of the balancers. At 32°C., these appear normal in vg flies.

3. Position and duration of the TEP.

Stanley and Harnly have repoted most of the extensive TEP studies in vg; Stanley at 17, 27, 30, and 31°C., and Harnly at 30, 31, 32, and 33°C. Their results are in fairly close agreement, and will be discussed together.

At 17°C., the TEP begins apparently with the second instar and extends probably through the early part of the third. At 27°C., the TEP begins possibly before the larva hatches from the egg, and appears to end midway through the second instar. There do not appear to be significant sexual differences in the length and position of the TEP at these two temperatures. At each temperature the TEP occupies approximately 30% of egg-larval development.

Beginning at 30°C. a strong sexual dimorphism manifests itself in the TEP, paralleling the dimorphism in the critical temperatures of phenotypic response. At all the higher temperatures the TEP appears to begin in both sexes with the molt that closes the second instar. At 30°C., the TEP ends, for males, about half way through the instar; for females, probably within the first quarter of the instar. At 31°C., the TEP includes the entire third instar for males, which is approximately 48 hours; for females, it includes only the first half of the instar, or 24 hours. At 32°C., puparium formation is greatly delayed, and only rarely do flies withstand the temperature long enough to pass through

this stage into pupation. The TEP ends in 48 hours for the males, as it did at 31°C. In females, however, the TEP at 32°C. shows no sign of completion at the end of 70 hours from its beginning. That is the limit of viable exposure for vg flies. At 33°C., the TEP in both sexes lasts the limit of the viable exposure, which is 50 hours, and does not appear to have ended at that time.

Harnly pointed out that the sexual dimorphism in phenotypic response to supra-critical temperatures can be formally explained on the basis of the similar differences in TEP length at these temperatures. There is a slight but consistent dimorphism in the 16 to 29°C. range, stronger in Stanley's data than in Harnly's. Males have slightly larger wings than females. This appears to be due to one or more modifiers in the X chromosome, as pointed out by Stanley and Riedel.

Li and Tsui, in their studies at 25 and 31°C., produced evidence that the TEP at 31°C. is not the only time when developmental activity of importance to the vg phenotype is taking place. They found that vg flies which spend the first larval instar, as well as the TEP, at 31°C. have considerably larger wings than do those flies which spend only the TEP at 31°C. Flies which spend the first instar at 31° C. and the TEP at 25°C. have the same sized wings as those which develop throughout at 25°C. The usual technique employed in TEP studies, therefore, is not sufficient to establish all the effective period of the vg phenotype.

4. Wing-size temperature relation.

Hersh and Ward, considering but three temperatures, concluded that the vg wing-size temperature relation is exponential. Harnly stated, however, that when one considers the entire temperature range, at least two logarithmic lines are necessary to fit the data. When the cases of combinations of dimorphos-vg and vg-pennant are also considered (Harnly and Harnly), several such lines are necessary. Harnly concluded that the logarithmic relation is not general in vg and its alleles.

5. The modifier, "dimorphos".

Harnly and Harnly reported the temperature response of the sex-linked modifying gene, "dimorphos" (dm), with vg (1935), and of vg and its allele, "pennant" (vg^p), (1936).

The gene, dm, does not manifest any visible effect by itself; but its presence in the genotype increases the size of vg wings at all temperatures. Its name originates in the fact that it affects the male wing much more than the female wing at temperatures below 32°C. The critical temperature for phenotypic response to temperature is shifted down to 25°C. in males and to 28°C. in females. In addition, a new and less extreme critical temperature appears for the males, probably between 16 and 18°C. At 32°C., the wings are of equal size in the two sexes, and 16% of them are like the wild-type wing, in both size and appearance.

6. The allele, "pennant".

The wings of pennant (vg^p) flies are normal in appearance at all temperatures. A few of them have terminal nicks and all of them seem to have weaker distal wing sections. In old flies, the distal portion of the wing is broken and ragged.

The response of vg^p to temperature is the same as that of wild-type throughout the viable range. Several investigators listed above have shown that the wild-type wing responds inversely, though only slightly, to temperature changes; and that females have somewhat larger wings than males at all temperatures. In both of these respects, the wild-type is the opposite of vg; and in both of them vg^p resembles the wild-type rather than vg, from which it arose as a mutation. At all temperatures, too, the absolute size of vg^p wings closely approximates that of the wild-type wing.

The response of vg/vg^p, however, is quite different from that of vg/wild-type. As shown by a number of investigators, heterozygous vg is phenotypically wild-type at all temperatures. The rate of decrease in wing size with temperature increase is retarded from that of the wild genotype. As a result, at 32°C. vg/wild-type is larger in area than the wild-type wing.

The wings of vg/vg^p are intermediate in phenotype, resembling "strap", at all but the higher temperatures. There is practically no change from 22 to 26°C.; but from there to 32°C. there is a sharp increase in size and a progressive change in phenotype. At 32°C. vg/vg^p is

fully the equal of wild-type or vg/vg^p in wing area; but a persistent terminal nick is present. (Since this terminal nick also appeared in a vg^p stock into which the modifiers of the vg stock had been crossed, it does not seem necessary to conclude with Harnly and Harnly that this effect is due to the vg gene.) In the combination of vg/vg^p, penna_nt seems to have shifted the original critical temperature of vg downward, and to have introduced a new and oppositely directed critical temperature at 22°C.

A strong sexual dimorphism exists in vg/vg^p. Below 30°C., the males have the larger wings; above 30°C., the females have the larger wings. This is analagous to the situation in vg/vg.

7. The allele, "No-wing".

Miss Gable (1938) has recently reported in abstract the temperature response of another allele of vg, "No-wing" (vg^{Nw}). This is a "No-wing" which I found among flies whose grandparents had been subjected to 36.5°C. temperature during part of their larval development (Plough and Ives 1935). It is more extreme than vg in its phenotypic and physiological effects at room temperatures. Heterozygous vg^{Nw} flies usually have terminally notched wings. Homozygous ones have apterous wings, smaller than vg; and the females are sterile.

The homozygous "No-wing" wing does not vary in appearance over the viable range, according to Miss Gable. The vg/vg^{Nw} wing is vg in appearance; but, like vg^{Nw}/vg^{Nw}

and unlike vg/vg, it does not vary in appearance over the viable temperature range. The vg^{p}/vg^{Nw} wing is intermediate in phenotype, and responds inversely to temperature changes throughout the range, the phenotype changing gradually from "strap" to vg. In this case, the response to temperature is that of vg^{p}. The vg^{Nw}/wild-type terminally notched wing is like wild-type in having an inverse relation to temperatures of the viable range; but it is consistently smaller than wild-type in area.

8. The dm-vg/vg^{p} combination.

Miss Lashin (1938) has reported in abstract form the response of the dm-vg/vg^{p} combination to temperatures in the range, 16 to 28°C. Wing length in this combination bears an inverse relation to temperature similar to that of wild-type and vg/vg. In males, the area of the wings is like that of vg^{p}/vg^{p}. In females, however, the area curve is U-shaped as in vg/vg^{p}, the area values being approximately twice those of vg/vg^{p} wings at corresponding temperatures. In phenotype the male wings may be wild-type; but they are usually nicked. In females, the phenotype is "antlered" or "strap". From the appearance of the area and length curves, and from the changes in phenotypes, she concludes that 28°C. is critical for dm-vg/vg^{p} flies.

The TEP's of dm-vg, vg^{p}, and vg^{Nw} and their heterozygotes have not been worked out. Nor have extensive embryological studies been made. One can not profitably suggest further relations from the above data alone.

9. Pattern in the vg series of alleles.

Harnly and Harnly stated that the order of succession of wing types appearing at increasing temperatures was essentially the same in vg, dm-vg, and vg/vg^p. Accordingly, they support the suggestion of several others that the wing develops according to a certain pattern, under the influence of the vg allelic series of genes. Li and Tsui also divided the wings which appeared in their work into several types, progressing from vg to normal type. But they emphasized that the types did not necessarily succeed each other as the number of hours spent by the larvae at 31°C. increased. They found no real evidence for a pattern of development in their vg studies.

10. Nature of action of vg and alleles.

Stanley advanced the hpothesis that vg produces an inactivating substance which acts upon wing-forming substance but is destroyed at higher temperatures. In vg/vg^p, however, the effect of temperature appeared with decreasing as well as with increasing temperatures. Harnly and Harnly concluded from this observation that vg does not produce such a substance.

Goldschmidt's (1935,1937) embryological studies indicated that at least the less extreme alleles of vg produce their effects apparently in the pupal stage by destroying wing tissue. In these alleles, the wing seems to be normal at the time when the pupal wing-sheath appears, early in pupal development. Subsequently, degeneration sets in. The amount of scalloping manifested in the adult wing appears

to depend upon the time of the beginning of this process. From this, he advanced the hypothesis that the more extreme vg alleles, and finally vg itself, push the beginning point of degeneration further and further back in development, so that there is more time for destruction of wing material before final differentiation has been accomplished. It is also his opinion that temperature acts upon the beginning point of this process similarly, moving it in one direction or another, as the phenotype indicates.

Li and Tsui counted the cells in a measured region of vg wings which had spent measured amounts of time at 31°C. in the TEP. They found that corresponding to the increase in wing size with increased time at 31°C., there was an increase in the number of cells in the wing, and a slight increase in the size of the cells. They also observed an increase in the size of the dorsal mesothoracic imaginal disc (from which the wing disc develops) in 31°C. vg larvae as compared to 25°C. larvae. From this they concluded that temperature may produce in the case of the vg wing a direct increase in growth as well as the degeneration process observed by Goldschmidt.

11. Relation of TEP to wing embryology.

Chen (1929) in his embryological studies on Drosophila reported that the dorsal mesothoracic bud first appears in the second instar period. In the third instar, it is already smaller in vg than in wild-type wing. The wing bud seems also to be delayed in vg in its differentiation from the dorsal mesothoracic bud. These effects were con-

siderably more pronounced in a "No-wing" stock. Stanley and Harnly stated that since the TEP of vg at the higher temperatures appears to begin early in the third instar, the effect of vg is probably upon the growth processes taking place at the time when the wing-forming area divides from the thoracic-forming area of the dorsal mesothoracic bud. The observations and conclusions of Li and Tsui are in accord with these views.

Auerbach (1936) has made a penetrating embryological study of the development of the same buds. She was able to show that the small dorsal mesothoracic bud is already present in newly hatched larvae. Poulson (1937) found the beginning of visible formation of the same bud about three-quarters of the way through embryonic development in histological sections of the 17 to 18 hour egg. Miss Auerbach traced the development of this bud throughout larval life and up to the formation of the wing sheath in the pre-pupal period. The first conclusive morphological difference at 26°C. between vg and wild-type wing she found to be just previous to puparium formation, when the wing begins to grow out from the wing-forming area of the dorsal mesothoracic disc. She admits the possibility of following the difference further back by histological methods.

Miss Auerbach made the important observation, simple though it is, that one does not have to associate the TEP with a contemporary and visible morphological process; that reactions may indeed be occuring which do not manifest themselves morphologically until as much later as the actual differentiation of the wing-forming area just

previous to pupation. At the same time, her observations and those of others, that the imaginal wing begins visible differentiation sometime previous to the hatching of larva from the egg and continues throughout larval and into pupal life makes it possible, for those who desire to do so, to associate practically any wing TEP they find with some visible, if not also differential, morphological process of development.

12. The TEP of the wild-type wing.

Stanley (1935) also determined the TEP for the wild-type wing. He found that it begins late in the third instar and continues probably to the middle of the pupal developmental period. This period is clearly later than the TEP of vg, and the duration is also considerably longer. It corresponds in time to the visible differentiation of the wing. The position of the TEP does not vary greatly over the viable temperature range.

13. Temperature effect on vg in D. virilis.

Nadler (1926) reported that in D. virilis, as in D. melanogaster, vg increases in size with increase in developmental temperature. The rate of increase seemed to follow the van't Hoff law for temperature effects on a chemical reaction, with an average Q_{10} value of 1.98. In the range, 12 to 20°C., Q_{10} was 2.44; and from 20 to 30°C. it was 1.51. Further studies of vg in this species have not appeared.

F. Bent Wing.

Metz (1923) reported briefly a study of the temperature response of the bent-wing phenotype in Drosophila melanogaster and D. virilis, in the range 9-12°, 16°, 23°, and 25°C. At the low temperatures, the effects of bent on the virilis eye ("roughening" of the facets) were considerably enhanced. Scutellar bristles were also misplaced or missing, and other new modifications appeared over the fly. The effects of the gene on the wings and legs seemed to be less extreme at the lower temperatures.

In melanogaster, bent did not affect the eyes at normal temperatures; but at the low temperatures a roughening effect appeared, less extreme than in virilis. In other respects the behavior of bent was much the same in the two species at the temperatures tested. The TEP seemed to be located in late larval and early pupal life in both species. Wild-type flies did not show any of the modifications of bent at these temperatures; and offspring of bent flies which manifested the extreme effects of cold were only ordinary bent at room temperatures. From this Metz concluded that temperature had only enhanced the effects of bent, and that modifying factors were probably not responsible for the phenomena. The time of the TEP corresponds to the time when extensive visible differentiation and expansion is occurring in the imaginal disc (Auerbach 1936).

G. Unequal Wings 17b.

Miss Auerbach (1936) studied the temperature response of "unequal wings 17b", a third chromosome mutant with variable manifestation. The percent of manifestation falls with the temperature of development, and is lower in males than in females. At 16°C. nearly all flies have normal wings. The TEP was determined by transfers between 16 and 27°C. and was found to occur late in the third instar, probably just preceding the prepupal period. From her studies on wing development, Miss Auerbach concluded that the decisive action of "17b" consists probably of an alteration in the process of wing-pouch formation.

H. Dumpy Wing.

Blanc and Child (1938) have reported in abstract the effects of brief exposure to 36.5°C. upon the expression of "dumpy" (dy) wings. In homozygous condition this gene produces a truncate wing at the usual room temperatures, and is not effective in heterozygous condition. Blanc and Child report that dy is an effective dominant when puae are exposed for 12 hours to 36.5°C. during the first day after pupation. Under these conditions, dy is more effective in males than in females. They observed in addition that wild-type flies show a clear but much weaker tendency to produce truncate wings under similar conditions. The TEP for this reaction corresponds to the time of dumpy wing degeneration in the pupal wing sheath as observed by Goldschmidt (1935).

I. Dichaete bristles.

Plunkett (1926) reported studies on Dichaete (D) in D. melanogaster. This gene prevents the formation of certain bristles of the scutellum and thorax. He was able to show by mean bristle numbers, by association constants between the bristles, and by observations on individual flies, that the effect of D is a regular pattern which centers at the presutural bristle and spreads in all directions. The lack of left-right correlation between the bristles of the pattern indicated that for the individual fly the pattern of the left half varies independently of the pattern of the right half. The simplest explanation seemed to be to assume that the D gene is somehow related to a substance which diffuses from the pattern center on each side of the fly during early development, and which, under the influence of D, prevents the formation of bristles.

Because of the high correlations between bristles of the pattern at 25°C., Plunkett confined his observations at other temperatures to one pair of these bristles, the posterior dorso-centrals (p dc). He found that the mean number (M) of p dc decreased steadily as the temperature increased from 15° to 30°C. The TEP varied but little in duration between 24 and 30°C., and it extended from early larval life until late in the pupal stage, close to the time when the bristles first become visible through the pupa case, a day or so before emergence of the imago. The change in M of p dc appeared to be proportional to the time spent at a given temperature at any time during this extended TEP.

When the Arrhenius u values of the thermal increment of the D reaction were plotted, they gave a straight line throughout the temperature range, with an average u value of 34,600 units. This value suggested the heat destruction of enzymes.

From further studies on the M p dc, Plunkett advanced an hypothesis to explain the action of D on the bristles of its pattern, and the effect of temperature upon this reaction. Extended theoretical kinetic considerations showed that the data from the p dc bristles at the several temperatures and from the pattern of bristles at 25°C. supported the hypothesis with admirable goodness of fit. Later unpublished data by Plunkett (information from Professor Sturtevant) on the other bristles at different temperatures indicated that these bristles did not behave similar to the p dc, and indicated the probability that the hypothesis developed from the p dc was much too simplified to be of use for the whole pattern at all temperatures.

J. Scute Bristles.

1. Introduction.

Payne (1920) first observed scute (sc), a sex-linked gene removing certain bristles of the scutellum, thorax, and head in D. melanogaster. He isolated it in the course of selection experiments on bristle number. He reported that a student of his, Froemming, found that the number of scutellar bristles present in the mutant was influenced by the developmental temperature. Flies raised

at 10 to 13°C. had more scutellar bristles than those raised at 23 to 26°C. The effect of the temperature seemed to be limited to the larval period.

2. Viable temperature response and TEP.

Child (1935, 1936) has published the results of an extended series of experiments with sc at temperatures ranging from 14 to 31°C. He considered all the bristles of the scutellum, thorax, and head. It was apparent not only that the mean frequencies of these bristles varied with temperature; but that the differences were not the same for each of the several bristles, either in amount or in direction. Some bristle frequencies reached maxima (100% present); some reached minima (0% present); some showed sharp rises in a relatively small temperature range; and some rose through part of the range and fell through the rest of it. The TEP for all bristles, however, when a population of flies was considered, occupied the same relative position in egg-larval development; namely, the last half of the third instar. Child was able to show experimentally and by theoretical considerations that for the individual fly the TEP is a much shorter period. For this study he used the ocellar bristle, whose response to temperature changes was the strongest of all the sc-affected bristles in his experiments. The TEP for this bristle proved to lie entirely between 89 and 97% of egg-larval life. It appeared to end just before the onset of puparium formation. Child did not conclude that the TEP is necessarily of exactly the same length for every bristle; but he believed

it to be apparent that for them too the TEP for the individual larva is much less than the TEP for the population of larvae in a given culture.

3. Temperature change during the TEP.

Child found that when larvae were transferred from one temperature to another during the TEP, the bristle frequencies were generally intermediate to the standard frequencies for the temperatures concerned. The frequencies of the anterior notopleural (an) and certain of the orbital (or) bristles were exceptions to this rule. When the change was from a low to a high temperature, the an bristle frequency was higher than either of the standard frequencies. When the temperatures were on opposite sides of 28°C., the or frequency was lower than either standard frequency. There was no apparent explanation for either of these anomalies.

4. Interpretation of the TEP.

Since the bristle frequency-temperature curves in Child's data vary so widely from bristle to bristle, and between the sexes, there is a strong suggestion that many reactions are involved. Child, however, pointed out that such a view is not the only possible one. The TEP's for all of the bristles occur in general in the same time period of development; and we are concerned, in each case, with the formation of a bristle. From this he argued that it is natural to assume that the same reaction is being effected at each bristle sight. He points out that it may be that for some bristles a temperature increase increases

the rate or duration of this reaction, relative to the rate or duration of development as a whole; while for other bristles a temperature increase decreases the rate or duration of the reaction, relative to the rate or duration of the development as a whole. If such differences in rate and duration can be shown, then the differential bristle-temperature curves can be formally explained; but Child's data are not critical on this point.

5. Relation of TEP to bristle embryology.

Robertson (1936) studied the pupal embryology of D. melanogaster. He found that pairs of trichogenic (hypodermal) cells, which later form bristles, first appear in the hypoderm about 27 hours after pupation. They develop into bristles within a very few hours. Child's TEP is prior to this period by about two days, and is therfore not related to any visible and strictly bristle embryological process.

6. Pattern in scute.

Contrary to Plunkett's demonstration of a pattern in Dichaete, Child found no evidence of a bristle pattern in sc; and this in spite of the fact that the frequency of a given bristle is constant in an inbred population as long as conditions remain constant. Observations on individual flies, and correlation and association coefficients for the several bristles showed clearly that the bristle frequencies are independent of each other over the viable temperature range. The temperature effect did not appear to be that of a pattern, either. The mean bristle frequencies changed

independently of each other as the temperature was changed.

Child pointed out that in the light of these facts, the seriation of sc-affected bristles according to their mean frequencies, as proposed by Sturtevant and Schultz (1931), is of no real significance; and that the complex five-division diffusion pattern proposed by Goldschmidt (1931) does not exist in sc-1. It is apparent from Child's results that when it is used in reference to sc, the term "pattern" carries no further meaning than "the bristles affected by sc".

7. Inadequacy of the sub-gene hypothesis.

Dubinin (1929), Dubinin and Friesen (1932), and a number of other people have studied the extensive series of sc alleles in reference to the relation of their bristle patterns. From these studies came the well known theory of the centers of the gene. It is possible to arrange all the bristles affected by the sc alleles in a linear seriation of such an order that under a given set of conditions each of the several alleles affects a certain number of the seriated bristles, in each case consecutively. The entire seriation was considered by these workers to represent the map of the basi-gene of the scute locus. Boundaries of the alleles, as indicated by the bristles they affected, were assumed to indicate the limits of subdivisions or centers of the basi-gene. A study of these alleles revealed twelve such centers, or sub-genes, in the sc basi-gene. Each allele contained one or more consecutive centers.

Child showed that when sc-1 flies are raised at

a lower temperature than was used in these studies of Dubinin and others, sc-1 affects bristles which are in some cases several centers removed from the supposed limits of sc-1, and fails to affect bristles in centers in which the sc-1 effect is strongest at higher temperatures. He found the same thing true in sc-5 (Child 1936). From this he concluded that the whole hypothesis of basi—gene and sub-genes, which had already been shown inadequate by the studies of Sturtevant and Schultz (1931) on the effects of extra sections of the X-chromosome on the bristle frequencies, is of no significance for an explanation of the relation of the sc alleles.

8. Temperature relations of sc-1 and sc-5.

Child (1936) reported studies with sc-1, sc-5, and their heterozygote at temperatures of the viable range. He found, as might be expected, that the bristle frequencies in sc-5 and sc-1/sc-5 showed varying types of changes along the temperature range. For each bristle, however, the direction of frequency change with progressive temperature change was the same in each of the three stocks, so far as it could be measured. And at all temperatures, for all bristles which differ in frequency between sc-1 and sc-5, the heterozygote showed intermediate bristle frequencies. A preliminary study indicated the probability that the TEP's and the duration of development differ between the three stocks.

9. The TEP at high temperature.

In 1935, I published a preliminary account of the results of a first series of experiments with sc-1, in which the data of the ocellar bristles showed that for brief exposures to 40°C., this bristle of sc-1 has a TEP which extends from early in the embryonic stage into the early pupal period. These experiments have been extended and will be presented and discussed in Section II of this report. It is sufficient to note here that they indicate the probability that there are indeed reactions going on throughout the bristle developmental period which affect the bristle frequency in sc, and which can be altered by a change in developmental temperature. By this fact, the data of these experiments make necessary a strictly qualified interpretation of the general meaning of viable temperature TEP studies relative to the time of differentiation of imaginal organ-forming material.

SECTION II

THE EXPERIMENTS WITH SCUTE AND WILD-TYPE

A. Introduction.

The general results of Child's viable temperature studies on sc were discussed in the last part of Section I of this report. In addition, a brief summary was given of an earlier report on the first of the high temperature experiments which will be discussed in this section.

After a preliminary investigation in the fall of 1934 had indicated that a short exposure of sc larvae to 40°C. produced changes in the male bristle frequencies of the subsequent adults, I planned a series of experiments to determine the bristle effects of such exposures during any one of a number of developmental time periods. These 40°C. experiments involved sc males from each of two matings; sc females x vermilion carnation (v car) males, and sc females x sc males.

A second series of experiments tested similarly the effects of 36°C. on bristle frequencies in sc males.

A third series of experiments, mostly at 36°C., tested the effects of such exposures on the bristle frequencies of females heterozygous for sc, and on the bristle frequencies of both sexes in a closely related wild-type stock.

In connection with these three series of experiments, data were also collected at 25 to 26°C. on the effects of environmental factors, such as larval density and the food preservative, Moldex, and of probably genetic modi-

fiers, on the bristle frequencies in sc males.

The presentation and discussion of these data will follow a discussion of the experimental materials and technique.

B. Materials and Methods.

1. Stocks.

The stocks used in these experiments were the standard scute-1 (sc) and vermilion carnation (v car) stocks of this laboratory. Each was inbred and selected for six generations before the beginning of the experiments late in 1934. The sc stock was selected for the absence of the bristles considered in these experiments (see below); and the v car was selected for the presence of the same bristles. Inbreeding consisted of using one or two pairs of selected sibs as parents in each generation. The wild-type stock was derived form these two mutant stocks. It was inbred without selection for 13 generations. After the period of inbreeding and selection, these stocks were continued generally by 20 pair matings and without extensive selection through the duration of the experiment. This amounted to three years for sc and v car, and to one and one-half years for the wild-type.

2. Relation of control and experimental flies.

Selection and inbreeding made each stock approximately isogenic. In order to mimimize the effects of residual heterogeneity and of possible new mutations, the experiments were planned so that comparison could be made between control and experimental flies which were sibs. A group of 15 to 20 pairs of flies, themselves sibs, were allowed to oviposit in each of a number of cultures. The cultures were then divided into control and experimental

lines. From 3 to 9 groups of sib parent flies were carried along simultaneously in this manner as one series.

The general procedure was to age the parents two days to attain high fecundity; to transfer them, then, to new cultures three times a day for the next six days; and finally, to use two of each day's cultures in experimental lines while keeping the third as control. This made it possible to compare sibs that were raised together in time and under similar environmental conditions. First comparisons were always made on that basis. However, when their bristle frequencies showed no significant differences, control cultures of two or more consecutive series were grouped as common controls for each of their experimental lines.

3. Incubators and temperature measurements.

The incubators used in the experiments were of the standard insulated type designed by Bridges (1932), using a toluene-mercury thermostat, employing a constantly running fan to minimize temperature variations, and containing pans of water to maintain a high humidity. The control incubator was capable of maintaining a temperature of 25°C. with a constancy of ±0.1°C. for any one shelf. Because of a difference between shelves, control and experimental cultures in each series were raised intermingled on the same shelf. Variation in room temperature and in the number of cultures in the incubator caused fluctuations in the incubator temperature which, through the three years of the experiment, with a few exceptions, amounted to not more than ±0.5°C. This made necessary a constant check of con-

trol bristle frequencies. Where there was a difference in the controls of two series which had to be compared, allowance has been made for the difference.

The temperature in the control incubator was read from the air on standardized thermometers. Tests showed that the culture temperature was generally above the air temperature, by as much as 0.5°C. when the incubator was crowded with cultures. In extreme cases, this difficulty was overcome by lowering the air temperature, and by critical use of the control bristle frequencies discussed above.

The exposures to high temperatures were made in two smaller incubators, similar in construction to the control incubator. Temperature was read from a thermometer placed in the middle of the culture food, where the larvae were observed to congregate. The thermometer remained in the food throughout the exposure and frequent checks of the temperature were made. Because of rather wide variations in temperature conditions in different parts of the incubators, it was possible to place the cultures in a warmer position until the food temperature had reached the desired level; and then to shift them to a cooler position to maintain the temperature. Under these conditions, the temperature rose from 25°C. to either 36 or 40°C. in about one hour.

The number of cultures exposed together varied between three and five in the 40°C. experiments and between six and nine in the 36°C. experiments. The temperature was read from one or more cultures of each exposure group. Tests

showed that there was a variation in the temperature between cultures of such a group. This amounted to about 0.5°C. in a group at 40°C. and to about 1° in a group at 36°C. The range of variation was larger than this for the many groups of an experiment. In all the groups of the sc x v car 40°C. experiment this variation was between 39.5 and 41.5°C. In the sc x sc experiment, it was between 39.5 and 40.5°C. In all of the 36°C. experiments, it was between 35 and 37°C.

At these higher extremes the food temperature tended to be lower than the air temperature. The difference was usually about 1° in the 40°C. series, and about 0.5° in the 36°C. series. This, it will be noted, is opposite to the tendency at 25°C.

4. The bristles and their classification.

Flies were classed for seven pairs of bristles, each pair being represented on the left and right sides of the fly. The bristles, with their abbreviations and locations, are as follows: posterior scutellar (ps) and anterior scutellar (as) on the scutellum; anterior notopleural (an) on the anterior lateral region of the thorax; postvertical (pv) on the medial posterior dorsal part of the head; ocellar (oc) on the top of the head, between the ocelli; and anterior and medial orbital (or) on the anterior dorsal rim of the eye. Because of an impossibility of distinction between the anterior and medial orbital bristles in sc-1, those two bristles were classed together as "orbital" (or).

In the sc x sc experiments, only sc males were classed. In experiments with sc females x v car males,

and in experiments with wild-type, bristles were counted in both sexes. The sc males were classed for the frequency of presence of the above bristles. The sc/v car females and both sexes of wild-type flies were classed for the absence of the same bristles. Each fly was recorded individually with respect to the presence or absence of each of the above bristles.

5. Calculation of bristle frequency.

As in Child's experiments, practically no correlation was found between the two bristles of any one pair, in both sc and wild-type flies, when the conditions were properly controlled at 25°C. Accordingly, since each half of the fly can have one bristle of each pair, and since the desired bristle frequency is that of the ratio,

$$\frac{\text{total observed number of x bristles}}{\text{Total possible number of x bristles}},$$

the "half-fly" unit, proposed by Plunkett (1926) and adopted by Child, has been used in these experiments.

As an example we may consider the oc frequency. Each fly represents two observations upon the oc bristle, one on the left side and one on the right side. Each half of the fly, then, is one observation upon the oc bristle; and the oc bristle frequency per half fly in a sample is that of the ratio,

$$\frac{\text{the number of oc bristles.}}{\text{Twice the number of flies}}$$

The maximum frequency is 1 and the minimum frequency is 0. The frequency therfore, can be stated as a percentage by multiplying the above ratio by 100.

All mean frequencies have been calculated in the above manner and tabulated as percentages.

6. Tabulation of differences in bristle frequencies.

Certain of the tables of data which will be discussed in this report represent summaries of bristle frequency changes produced by temperature treatment. As a convenient method for tabulating these summaries, I have chosen the value of the ratio,

$$\frac{\text{difference between control and experimental frequency}}{\text{control frequency}}$$

which I have named, the "Coefficient of Change". The sign of the coefficient indicates the direction of the change. It can be seen that when the control frequency is 0 and the experimental frequency is greater than 0, the value of the coefficient is infinity. Since the purpose of the coefficient is to make possible an easy and direct comparison of degrees of change in bristle frequencies, in the one or two instances where such a situation as the above has arisen in the data and the increase appeared to be of some significance, the control frequency has been arbitrarily put at 0.1. Infinity, however, represents the maximum of increase as expressed by the coefficient of change. Similarly, -1 represents the maximum of decrease, and is observed when the experimental frequency is 0 and the control frequency is greater than 0. When there is no difference between the control and the experimental frequencies, the value of the coefficient of change is 0.

7. Statistical formulae.

Standard error has been employed in all data considerations for estimating the probability of differ-

ence between mean bristle frequencies. The approximate standard error (s) of a mean bristle frequency is given by

$$s = \pm\sqrt{\frac{p(1-p)}{n}}$$

where p is the mean bristle frequency and n is the number of half-fly observations. The standard error of a difference (s_d) between two mean bristle frequencies, p_1 and p_2, is given by

$$s = \pm\sqrt{s_1^2 + s_2^2}$$

where s_1 and s_2 are the standard errors of p_1 and p_2 respectively. In general, a difference has been considered significant only when it equals or exceeds twice its standard error. A difference which is smaller than that amount will occur by chance alone more often than once in twenty comparisons between samples drawn from a uniform population.

For an estimate of correlation (r) between bristles I have used the formula

$$r = \frac{S\,(xy)}{\sqrt{S\,(x^2) \cdot S\,(y^2)}}$$

where x and y refer to the bristles under consideration.

The "t" test of Fisher (1928) has been used to estimate the significance of the deviation of r from 0. The value of t is determined from the formula

$$t = \frac{r}{\sqrt{1 - r^2}} \cdot \sqrt{n - 2}$$

where n is the number of pairs of observations (in this case the number of flies) in the sample, and r is the cor-

relation coefficient. When the size of the sample exceeds 100 flies, r may be considered as significantly different from 0 when t equals or exceeds 2, since 2 is approximately the "1 in 20" level. The more that t exceeds 2, the larger the chance of significance in the deviation from 0.

C. The Effects of Environmental Factors and Genetic Modifiers.

1. The independence of bristles.

It has already been noted that Child found a complete absence of pattern relationship in the sc-affected bristles. He found this by observations on individual flies, by absence of correlations between the bristles, and by the small size of calculated association coefficients. So far as they go, my data are in complete agreement with his on this point. Checking of the individual flies indicated clearly that only in the special case of the anterior and medial orbital bristles was there any association between the bristles with which I have concerned myself. Child reported a complete negative association between these two bristles and ovserved no instance of their simultaneous appearance on the same side of the fly. In some 500,000 half-fly observations, I found only one such instance.

Extensive correlation coefficient calculations were made from the male control data of the 1936-1937 experiments with sc x sc and sc x v car. These correlations included all the two-by-two combinations of the six bristles: ps, as, an, pv, oc, and or. The value of r ranged between -0.010 and +0.040 in the sc males from sc x sc; and between -0.007 and +0.074 in sc males from sc x v car. While the t values exceeded 2 for the larger positive correlations, because of the large numbers of

flies observed, the correlations are too small to be of significance for these experiments. There is not the tendency for covariance among the bristles that one would expect if a definite pattern realationship obtained among them.

2. Effects of crowding of larvae.

Plunkett (1926) included a table of data to show that increasing mean number of larvae per culture caused a decided decrease in the mean number of posterior dorsocentral bristles in the subsequent Dichaete adults. He attributed the effect to a change in food such that the later larvae from eggs oviposited over a period of several days were raised under different conditions than were their earlier sibs. The effect disappeared when an egg-laying period of one day or less was used.

Child published no data on crowding effects in sc. He used short laying periods, however, and kept the larval density of a culture low.

Early in the course of my experiments it was noted that larval density affected bristle frequency, even when the egg-laying period did not exceed 12 hours. In addition, it was apparent that not all bristles were affected similarly.

In Tables 1 through 7 are presented samples of the control temperature data from sc males to demonstrate these facts. Each table is a summation of a number of consecutive series which did not vary significantly within themselves. The cultures comprising each table have been

TABLE 1.

sc ♀♀ x v car ♂♂ - Nov. 1934 25°C.

Size	Obser.	ps	as	an	pv	oc	or
76 (41 - 99)	1274	0.1 ±0.1	1.7 ±0.4	0.6 ±0.2	0.9 ±0.3	4.2 ±0.5	7.9 ±0.8
147 (108 -190)	4398	0.7 ±0.1	2.8 ±0.2	0.3 ±0.1	0.7 ±0.1	3.9 ±0.3	4.2 ±0.3
239 (209 - 290)	2874	0.7 ±0.2	3.7 ±0.4	0.5 ±0.1	0.7 ±0.2	3.4 ±0.3	3.0 ±0.3

TABLE 2.

sc ♀♀ x v car ♂♂ - May 1935 25°C.

Size	Obser.	ps	as	an	pv	oc	or
141 (72 - 189)	1388	0.3 ±0.1	2.6 ±0.4	0.2 ±0.1	0.4 ±0.2	3.4 ±0.5	7.1 ±0.7
255 (203 - 294)	2762	0.65 ±0.15	4.3 ±0.4	0.4 ±0.1	0.5 ±0.1	2.6 ±0.3	4.6 ±0.4
375 (315 - 421)	1848	1.2 ±0.3	4.7 ±0.5	0.9 ±0.2	0.65 ±0.2	3.3 ±0.4	4.3 ±0.5

TABLE 3.

sc ♀♀ x sc ♂♂ - Oct. 1936 25°C.

Size	Obser.	ps	as	an	pv	oc	or
125 (57 - 182)	1916	0	0.4 ±0.1	0.6 ±0.2	0.6 ±0.2	3.9 ±0.4	17.0 ±0.9
226 (183 - 275)	3388	0.06 ±0.04	0.3 ±0.1	0.5 ±0.1	0.7 ±0.1	4.4 ±0.3	12.3 ±0.6
315 (295 - 366)	2788	0.04 ±0.04	0.4 ±0.1	0.3 ±0.1	0.8 ±0.2	2.1 ±0.3	6.6 ±0.5

TABLE 4.

sc ♀♀ x sc ♂♂ - Feb. 1937 25°C.

Size	Obser.	ps	as	an	pv	oc	or
112 (72 - 139)	2096	0	0.5 ±0.1	0.8 ±0.2	1.4 ±0.3	3.1 ±0.4	16.3 ±0.8
173 (143 - 199)	2102	0.2 ±0.1	0.8 ±0.2	0.4 ±0.1	1.4 ±0.3	2.8 ±0.4	11.0 ±0.7
248 (201 - 302)	5636	0.04 ±0.03	0.8 ±0.1	0.8 ±0.1	0.9 ±0.1	3.5 ±0.3	9.4 ±0.4

TABLE 5.

sc ♀♀ x sc ♂♂ - March 1937 25°C. 1% Moldex

Size	Obser.	ps	as	an	pv	oc	or
110 (82 - 139)	1360	0.1 ±0.1	1.1 ±0.3	0.8 ±0.2	0.9 ±0.3	2.5 ±0.4	9.9 ±0.8
171 (140 - 198)	6190	0.1 ±0.04	1.0 ±0.1	1.0 ±0.1	1.1 ±0.1	2.8 ±0.2	6.9 ±0.3
242 (200 - 296)	5978	0.2 ±0.06	1.2 ±0.1	1.4 ±0.2	0.8 ±0.1	3.8 ±0.2	4.8 ±0.3

TABLE 6.

sc ♀♀ x sc ♂♂ - April 1937 25.5°C. o.7% Moldex

Size	Obser.	ps	as	an	pv	oc	or
108 (68 - 134)	3538	0	0.1 ±0.05	0.8 ±0.2	0.7 ±0.1	4.2 ±0.3	13.5 ±0.6
171 (142 - 196)	9122	0.09 ±0.03	0.3 ±0.06	0.8 ±0.1	0.7 ±0.1	5.0 ±0.2	7.8 ±0.3
240 (198 - 286)	7282	0.04 ±0.02	0.6 ±0.1	0.9 ±0.1	0.4 ±0.1	5.0 ±0.3	5.1 ±0.3

TABLE 7.

sc ♀♀ x v car ♂♂ - May 1937 26°C. 0.7% Moldex

Size	Obser.	ps	as	an	pv	oc	or
196 (152 - 218)	1316	0.8 ±0.2	4.3 ±0.5	1.6 ±0.3	2.0 ± 0.4	10.6 ±0.9	3.3 ±0.5
278 (268 - 288)	1918	1.0 ±0.2	6.6 ±0.6	3.0 ±0.4	1.4 ±0.3	10.6 ±0.7	2.3 ±0.3
365 (324 - 420)	1764	1.7 ±0.3	8.2 ±0.7	3.6 ±0.4	1.1 ±0.3	11.8 ±0.8	2.7 ±0.4

grouped according to "size"; i. e., the number of flies recorded per culture. Included in each table are: the mean culture size, including in parentheses the sizes of the smallest and largest cultures in the group; the number of male half-fly observations; and the mean percentile bristle frequencies, with their standard errors, for each of the six bristles considered in this work. At the head of each table is a statement of pedigree which includes the mating, the date in year and month, and the temperature and food conditions (discussed later) of the included cultures.

An examination of Tables 1 through 7 shows that some of the bristle frequencies changed as the mean culture size increased. A considerably larger amount of control data from conditions similar to those of Tables 1 through 4 amplify the data of these tables. From a consideration of all the data one can make the following general statements concerning the relation of the several bristles to larval density. In the matings of sc x v car, the ps, as, and an bristles tend to increase in frequency as the mean culture size increases; the or bristles tend to decrease; and the pv and oc bristles show practically no change with change in size of culture. In the sc x sc matings, the only clear change is a decrease in the or frequency with increasing culture size.

Left-right correlation coefficients were calculated for each of the six pairs of bristles in all of the control data. In no case was there any tendency for a change in the value of r for any pair of bristles as the

mean culture size increased. This indicates the probability that in these experiments the effect of increasing larval density was on the entire culture population, rather than on a part of it. This is probably due to the short egg-laying periods used in these experiments.

Correlation coefficients were also calculated for each of the two-by-two bristle comparisons in these data. In no case was there a correlation above 0.1. It seems probable therefore that there was no significant tendency for covariance among the affected bristles.

The data represented in these tables indicate clearly that larval density is a factor which influences some of the mean bristle frequencies in sc males. Accordingly, in the data to be presented on the effects of exposures to 40 and 36°C., I have made comparisons between experimental and control series with as nearly the same mean culture size as was possible.

3. Effects of Moldex.

In February of 1937, the food formula of this laboratory was changed to include a mold preventitive known by the trade name, "Moldex" (Glyco Products Co., New York). It is a methyl ester of parahydroxybenzoic acid. In this laboratory it is kept in a stock solution of 10 g. of Moldex in 90 cc. of 95% ethyl alcohol. This solution of Moldex constituted 1% of the food for a month, after which time the proportion was lowered to 0.7%.

The cultures of Tables 5 through 7 contained Moldex, as indicated in the table headings. Tables 4 and 5 are

expecially instructive concerning the effect of Moldex on the bristle frequencies. Table 4 cultures contained no Moldex, while Table 5 cultures contained the 1% Moldex formula. So far as is known other conditions were alike for the two groups of cultures. The main effect of the Moldex, as revealed in these two tables, is on the or Bristle, whose frequency is clearly lower in the Moldex series. In this mating, Moldex does not appear to affect the other bristles significantly.

The data in Tables 6 and 7 show the effects of the 0.7% Moldex. There are differences in the temperature conditions in these two tables which make necessary certain corrections before the data can be compared with those of lower temperature series. Occasional small series of the non-Moldex experiments had temperatures comparable to these. From them it appears that in sc x sc the effect of the slightly higher temperature was mostly on the oc bristle, and that the main effect of the 0.7% Moldex was to decrease the frequency of the or bristle. There does not appear to be a significant difference between the effects of the two strengths of Moldex.

The effect of Moldex on the males from sc x v car is more extensive than it is on the males from sc x sc. This is brought out in the data of Table 7. From series raised at similar temperatures in the earlier non-Moldex experiments it was noted that the effect of the temperature is manifested in decreases in the ps, as, and pv bristles; and in increases in the an, oc, and or bristles, the oc

increase being most marked. A comparison of the data of Table 7 with those of Table 2, its nearest control in point of time, reveals that the Moldex seems to have increased the ps, as, and pv frequencies, and to have decreased the or frequency. In each case, the frequency change is the opposite from what would have been expected from the effects of the temperature difference alone. This probably means that in the sc x v car mating, the effects of Moldex are even more pronounced than indicated in these data of Table 7.

Left-right correlation coefficients of the affected bristles were compared in Moldex and non-Moldex series. There were no consistent differences between the two series. This indicates that the effect of the Moldex was upon the entire population. Two-by-two correlation coefficients of the different bristles were calculated and were generally less than 0.1, indicating that the bristles did not tend to vary together under the influence of Moldex.

With a view to indicating a possible explanation for the effects of Moldex upon the bristle frequencies of sc, a rough test was made of the effects of Moldex upon the growth of yeast. A glucose-yeast extract media was made up to contain, in one culture, 1% of the Moldex solution, and in another culture of the same volume, 1% of 95% alcohol (the base of the stock Moldex solution). The cultures were then innoculated with equal amounts of a yeast suspension. At the end of 10 days the dry weight of the yeast in the non-Moldex cultures was slightly more than twice that

in the 1% Moldex culture, indicating that Moldex decreases the growth rate of yeast. This test suggests the possibility that the effect of Moldex on the bristle frequency is indirect, and that the lower per capita supply of yeast for larval consumption may be the causal factor.

The effects of Moldex and larval density, it can be seen, are in the same direction on at least all but the pv bristles. Accordingly, we may offer as a suggestion the possibility that the effect of larval crowding is likewise due to a decrease in the per capita supply of yeast for larval consumption.

Child and Albertowicz (1937) reported in abstract that larvae developed "more slowly" on food treated with Nipagen, which is ethyl para hydroxy benzoate, and, as can be seen, is closely related to Moldex. Increasing the concentration of Nipagen lengthened the time of development. They found that vg flies which were raised on Nipagen developed larger wings resembling the less extreme alleles of vg raised under normal conditions. The size of the wing increased as the time of development was prolonged by the use of Nipagen.

No measurement has been made of the effect of Moldex on the length of larval development, excepting to note that there did not appear to be any large difference in the time of pupation when Moldex was added to the food. The data are not critical, however, for differences of less than half a day.

One is tempted to suggest, even in the absence of data, that the effects of Moldex and Nipagen may both be

upon the yeast, a lower per capita quantity of which delays larval development and gives the vg and sc genotypes a correspondingly longer time to affect the adult characters. Carefully controlled experiments should indicate whether or not the situation is as simple as this.

4. Effects of genetic modifiers.

In his selection experiments with low and high bristle frequency strains of sc, Payne (1920) found at least three, and probably more, genetic modifiers of the effect of sc on the scutellar bristles. One modifier was sex-linked (near miniature) and the others were autosomal. In the data of Tables 1 through 7 there is evidence for the presence of genetic modifiers in my experimental stocks. Additional evidence will be pointed out from the 40 and 36°C. series.

A comparison of the data from sc x v car with those of sc x sc in Tables 1 through 7 shows that three of the bristle frequencies differ between the two matings. In the sc x v car mating, the ps and as frequencies are higher and the or frequency is lower than comparable frequencies in the sc x sc mating. No attempt has been made to localize the factor or factors responsible for these differences.

Since both sc and v car were selected and inbred for several generations previous to the beginning of the experiment each stock should have been practically homozygous for its modifiers of the selected bristles. The calculation of left-right correlation coefficients showed that

this condition had been realized in the sc stock and for all bristles excepting the as bristle in the v car stock. In the sc male as bristle data from the sc x v car mating there was a persistent though small positive left-right correlation in both the 1934-1935 and the 1937 series. The value of r ranged between +0.1 and +0.2 (with t values of from 4 to 9) in all the series of this mating. This was not the case in the sc x sc mating where the value of r was sometimes positive and sometimes negative, and rarely as large as 0.1 in any case, It seems probable therefore that in this one case the v car stock was not homozygous for a sc modifier. Since the stock did not appear to change with time in respect to the proportion of flies carrying the modifier, the fact of presence does not complicate the comparison of control and experimental data.

It is important to know if the sc and v car stocks remained fairly constant genetically during the course of the experiments. Data bearing on this question are those of Tables 1 and 2, and 3 and 4. In the first case, the data represent series of sc x v car flies which were raised several months apart. In the second case, the data represent sc x sc flies which were raised several months apart.

It appears at first that in both cases there was a small change in the stocks with regard to the or bristle frequency. The or bristle frequency appears to have increased with time in the sc x v car mating, and to have decreased with time in the sc x sc mating. If this were due to a change in genetic constitution of the stocks, one would expect, assuming the change to have been caused by a mutation

or mutations, that only a relatively small proportion of the stock in each case would be responsible and that, accordingly, the left-right correlations would show evidence of the change. As a matter of fact, the left-right correlations are not significantly different between the two series in each mating. The r values for the or bristles are +0.010 and+0.015 for the data of Tables 1 and 2, respectively; and +0.056 and +0.026 for the total data of Tables 3 and 4 respectively. It seems probable, therefore, that the small changes in the or frequency in each case represent some slight and unknown change in environmental conditions which has affected the entire population.

The data of the Moldex series of Tables 6 and 7 are not directly comparable with each other because of the difference in developmental temperature discussed above. But when allowance is made for that difference, these data indicate that the bristle frequency differences between the two matings are in the same direction as in the non-Moldex series. There is the additional possibility that the differences were accentuated by the Moldex; but more critical data than these are necessary to establish this point.

5. Summary and Conclusions.

(1) There is no evidence in the data from the 25 to 26°C. series of these experiments that a pattern relationship exists between the bristles affected by sc, or that they tend to vary together in frequency of presence.

(2) Larval density influences some of the bristle frequencies of sc males under different constant environmental and genetic conditions, increasing the ps, as, and an frequencies in sc x v car matings, and decreasing the or frequencies in both sc x v car and sc x sc matings.

(3) The presence of 0.7% or of 1% of Moldex in the food increases the frequencies of ps, as, and pv bristles in the sc x v car mating and decreases the frequencies of or bristles in both sc x v car and sc x sc matings.

(4) The differences in bristle frequencies in sc males from sc x v car as compared to those in sc males from sc x sc suggest that the sc and v car stocks differ genetically in modifiers of sc-affected bristles.

(5) The sc and v car stocks do not appear to have changed significantly in genetic constitution during the course of the experiments, so far as the bristle frequencies are concerned.

(6) The results of these studies on environmental and genetic factors demonstrate the necessity of controlled conditions in experimental work with sc bristle frequencies.

D. The 40°C. Experiments with sc Males.

1. General plan of the experiments.

Two experiments have been carried out to test the effects of brief exposures to 40°C. on the bristle frequencies in sc males. The matings of these series were, in the first experiment, sc females x v car males, and in the second experiment, sc females x sc males. The general control of conditions has already been discussed, and sample control data have been presented from each of these experiments (Tables 1 through 6). The only significant difference in condition between control and experimental lines was in the exposure to the high temperature at a given period in development.

The exposures to 40°C. were made during six developmental periods: 0 to 10 hours, 20 to 30 hours, 48 to 58 hours, 72 to 82 hours, 96 to 106 hours, and 120 to 130 hours after oviposition. The ten hours in each period is the maximum sum of the egg-laying and exposure periods in the experimental cultures in each case.

No attempts were made to determine anatomically the exact morphological period represented in each of the above time periods. It was noted, however, that the majority of the larvae of these series, in not too crowded control cultures (under 200 larvae), pupated between 110 and 120 hours after oviposition. A number of investigators, among them Dobzhansky and Duncan (1933) in this laboratory, have reported a pupation time for D. melanogaster more or

less similar to the above at temperatures of 25 to 26°C. On the basis of these investigations on morphological developmental periods, the above time periods of my experiments may be designated approximately as: the embryonic period; the first larval instar; the second instar; the first half of the third instar; the second half of the third instar; and the early pupal period. From the studies of Robertson (1936) it appears that these periods cover practically the entire developmental period of the bristles.

A graded series of exposures to 40°C. were made during the above periods. The hour necessary to raise a culture temperature from 25°C. to 40°C. will be referred to as "a". Including this hour, the series of exposures used were: "a", ½, 1, 1½, 2, 2½, and 3 hours.

In the sc x v car experiment all of these exposures were used in the larval developmental periods; but in the embryonic and young pupal periods the half-hour exposures were omitted.

In the sc x sc experiment, generally only the 1, 2, and 3 hour exposures were used.

2. The data from sc x v car.

Tables 8 through 13 present the bristle data of sc males from the mating, sc females x v car males. The experiments were done in the school year of 1934-1935. At the head of each table is a statement of the mating, the exposure temperature, and the developmental period, morphologically and in hours from oviposition, during which the exposure was made. Included in the tables are: the length

TABLE 8.

sc x v car - 40°C. - Embryo, 0 to 10 Hours Development

Group	Size	Obser.	ps	as	an	pv	oc	or
"a";1;2	42	1216	0.1 ±0.1	2.4 ±0.4	0.8 ±0.3	0.6 ±0.2	11.3 ±0.9	12.8 ±1.0
Control	141	1388	0.3 ±0.1	2.6 ±0.4	0.2 ±0.1	0.4 ±0.2	3.4 ±0.5	7.1 ±0.7
Difference			-0.2 ±0.1	-0.2 ±0.6	+0.6 ±0.3	+0.2 ±0.3	+7.9 ±1.0	+5.7 ±1.2

TABLE 9.

sc x v car - 40°C. - First Instar; 20 to 30 Hours -

Group	Size	Obser.	ps	as	an	pv	oc	or
"a"	192	4286	0.5 ±0.1	2.5 ±0.2	1.1 ±0.2	0.5 ±0.1	5.2 ±0.3	4.7 ±0.3
Control	203	4658	0.5 ±0.1	3.0 ±0.3	0.9 ±0.1	0.3 ±0.1	4.2 ±0.3	3.3 ±0.3
Difference			0	+0.5 ±0.4	+0.2 ±0.2	+0.2 ±0.1	+1.0 ±0.4	+1.4 ±0.4
½	133	1844	0,3 ±0.1	3.4 ±0.4	0.2 ±0.1	1.1 ±0.2	9.8 ±0.7	7.5 ±0.6
Control	147	4398	0.7 ±0.1	2.8 ±0.2	0.3 ±0.1	0.7 ±0.1	3.9 ±0.3	4.2 ±0.3
Difference			-0.4 ±0.1	+0.6 ±0.4	-0.1 ±0.1	+0.4 ±0.2	+5.9 ±0.8	+3.3 ±0.7
1	78	1514	0.2 ±0.1	2.6 ±0.4	0.6 ±0.2	0.8 ±0.2	7.0 ±0.7	7.4 ±0.7
Control	76	1274	0.1 ±0.1	1.7 ±0.4	0.6 ±0.2	0.9 ±0.3	4.2 ±0.5	7.9 ±0.8
Difference			+0.1 ±0.1	+0.9 ±0.6	0	-0.1 ±0.4	+2.8 ±0.9	-0.5 ±1.0

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TABLE 9. (Cont.)

Group	Size	Obser.	ps	as	an	pv	oc	or
1½	114	1440	0.3 ±0.1	2.0 ±0.4	0.9 ±0.3	0.4 ±0.2	5.8 ±0.6	9.2 ±0.8
Control	112	5672	0.4 ±0.1	2.3 ±0.2	0.5 ±0.1	0.8 ±0.1	4.0 ±0.3	6.0 ±0.3
Difference			-0.1 ±0.1	-0.3 ±0.5	+0.4 ±0.3	-0.4 ±0.2	+1.8 ±0.7	+3.2 ±0.9
2	127	1474	0.5 ±0.2	3.7 ±0.5	0	0.1 ±0.1	4.7 ±0.6	5.8 ±0.6
Control	147	4398	0.7 ±0.1	2.8 ±0.2	0.3 ±0.1	0.7 ±0.1	3.9 ±0.7	4.2 ±0.3
Difference			-0.2 ±0.2	+0.9 ±0.5	-0.3 ±0.1	-0.6 ±0.1	+0.8 ±0.9	+1.6 ±0.7
2½;3	123	1540	0.2 ±0.1	0.7 ±0.2	1.0 ±0.3	0.1 ± 0.1	15.5 ±0.9	8.1 ±0.7
Control	166	2214	0.3 ±0.1	0.6 ±0.2	1.1 ±0.2	0.1 ±0.1	8.8 ±0.6	5.1 ±0.5
Difference			-0.1 ±0.1	+0.1 ±0.3	-0.1 ±0.4	0	+6.7 ±1.1	+3.0 ±0.9

TABLE 10.

sc x v car - 40°C. - Second Instar; 48 to 58 Hours -

Group	Size	Obser.	ps	as	an	pv	oc	or
"a"	131	894	0.3 ±0.2	1.5 ±0.4	0.2 ±0.2	0.2 ±0.2	3.1 ±0.6	9.3 ±1.0
Control	158	1800	0.4 ±0.1	1.7 ±0.3	0.3 ±0.1	0.4 ±0.1	2.8 ±0.4	5.1 ±0.5
Difference			-0.1 ±0.2	-0.2 ±0.5	-0.1 ±0.2	-0.2 ±0.2	+0.3 ± 0.7	+4.2 ±1.1
½	139	1474	0	1.4 ±0.3	0.1 ±0.1	0.1 ±0.1	5.2 ±0.6	8.7 ±0.7
Control	158	1800	0.4 ±0.1	1.7 ±0.3	0.3 ±0.1	0.4 ±0.1	2.8 ±0.4	5.1 ±0.5
Difference			-0.4 ±0.1	-0.3 ±0.4	-0.2 ±0.1	-0.3 ±0.1	+2.4 ±0.7	+3.6 ±0.9
1	118	978	0.3 ±0.2	1.5 ±0.4	0	0	5.3 ±0.7	9.3 ±0.9
Control	158	1800	0.4 ±0.1	1.7 ±0.3	0.3 ±0.1	0.4 ±0.1	2.8 ±0.4	5.1 ±0.5
Difference			-0.1 ±0.2	-0.2 ±0.5	-0.3 ±0.1	+0.4 ±0.1	+2.5 ±0.8	+4.2 ±1.0

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TABLE 10. (Cont.)

Group	Size	Obser.	ps	as	an	pv	oc	or
$1\frac{1}{2}$	145	860	0.1 ±0.1	1.2 ±0.4	0.1 ±0.1	0	10.2 ±1.0	9.5 ±1.0
Control	141	1838	0.4 ±0.1	2.7 ±0.4	0.6 ±0.2	0.2 ±0.1	4.2 ±0.5	6.0 ±0.6
Difference			-0.3 ±0.1	-1.5 ±0.6	-0.5 ±0.2	-0.2 ±0.1	+6.0 ±1.1	+3.5 ±1.2
2	142	2352	0.2 ±0.1	1.2 ±0.2	0.4 ±0.1	0.04 ±0.04	13.3 ±0.7	9.1 ±0.6
Control	150	3638	0.4 ±0.1	2.2 ±0.2	0.4 ±0.1	0.30 ±0.09	3.5 ±0.3	5.6 ±0.4
Difference			-0.2 ±0.1	-1.0 ±0.3	0	-0.26 ±0.10	+9.8 ± 0.8	+3.5 ±0.7
$2\frac{1}{2}$	150	1290	0.6 ±0.2	3.0 ±0.5	1.0 ±0.3	0.3 ±0.1	8.8 ±0.8	9.3 ±0.8
Control	141	1838	0.4 ±0.1	2.7 ±0.4	0.6 ±0.2	0.2 ±0.1	4.2 ±0.5	6.0 ±0.6
Difference			+0.2 ±0.2	+0.3 ±0.6	+0.4 ±0.4	+0.1 ±0.1	+4.6 ±1.0	+3.3 ±1.0
3	130	1244	0	1.1 ±0.3	0.3 ±0.2	0.1 ±0.1	7.2 ±0.7	8.4 ±0.8
Control	150	3638	0.4 ±0.1	2.2 ±0.2	0.4 ±0.1	0.3 ±0.1	3.5 ±0.3	5.6 ±0.4
Difference			-0.4 ±0.1	-1.1 ±0.4	-0.1 ±0.2	-0.2 ±0.1	+3.7 ±0.8	+2.8 ±0.9

TABLE 11

sc x v car - 40°C. - Third Instar; 72 to 82 Hours -

Group	Size	Obser.	ps	as	an	pv	oc	or
"a"	95	920	0.4 ±0.2	2.0 ±0.5	0.8 ±0.3	0.1 ±0.1	11.8 ±1.1	8.8 ±0.9
Control	117	1342	0.3 ±0.1	1.9 ±0.4	0.2 ±0.1	0.2 ±0.1	6.1 ±0.7	7.2 ±0.7
Difference			+0.1 ±0.2	+0.1 ±0.6	+0.6 ±0.3	-0.1 ±0.1	+5.7 ±1.3	+0.6 ±1.1
½	105	1116	0.3 ±0.2	1.5 ±0.4	1.1 ±0.3	0	10.4 ±0.9	7.0 ±0.8
Control	117	1788	0.3 ±0.1	2.3 ±0.4	0.2 ±0.1	0.2 ±0.2	4.6 ±0.5	6.5 ±0.6
Difference			0	-0.8 ±0.6	+0.9 ±0.3	-0.2 ±0.1	+5.8 ±1.0	+0.5 ±1.0
1	136	832	0.1 ±0.1	3.0 ±0.6	0.7 ±0.3	0	8.4 ±1.0	2.8 ±0.6
Control	134	1834	0.3 ±0.1	2.6 ±0.4	0.2 ±0.1	0.4 ±0.1	3.3 ±0.4	6.8 ±0.6
Difference			-0.2 ±0.1	+0.4 ±0.7	+0.5 ±0.3	-0.4 ±0.1	+5.1 ±1.1	-4.0 ±0.9

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TABLE 11 (cont.)

Group	Size	Obser.	ps	as	an	pv	oc	or
1½	103	916	0.3 ±0.2	1.7 ±0.4	0.9 ±0.3	0	9.3 ±1.0	5.4 ±0.7
Control	117	1342	0.3 ±0.1	1.9 ±0.4	0.2 ±0.1	0.2 ±0.1	6.1 ±0.7	7.2 ±0.7
Difference			0	-0.2 ±0.6	+0.7 ±0.3	-0.2 ±0.1	+3.2 ±1.2	-1.8 ±1.0
2	105	1280	0.2 ±0.1	1.3 ±0.3	0.8 ±0.3	0	11.0 ±0.9	5.4 ±0.6
Control	117	1342	0.3 ±0.1	1.9 ±0.4	0.2 ±0.1	0.2 ±0.1	6.1 ±0.7	7.2 ±0.7
Difference			-0.1 ±0.1	-0.6 ±0.5	+0.6 ±0.3	-0.2 ±0.1	+4.9 ±1.2	-1.8 ±0.9
2½	111	1290	0.2 ±0.1	3.2 ±0.5	0.9 ±0.3	0.4 ±0.2	9.2 ±0.8	5.5 ±0.6
Control	117	1342	0.3 ±0.1	1.9 ±0.4	0.2 ±0.1	0.2 ±0.1	6.1 ±0.7	7.2 ±0.7
Difference			-0.1 ±0.1	+1.3 ±0.6	+0.7 ±0.3	+0.2 ±0.2	+3.1 ±1.1	-1.7 ±0.9
3	137	1242	0.2 ±0.1	2.9 ±0.5	0.4 ±0.2	0	10.1 ±0.9	5.1 ±0.6
Control	117	1342	0.3 ±0.1	1.9 ±0.4	0.2 ±0.1	0.2 ±0.1	6.1 ±0.7	7.2 ±0.7
Difference			-0.1 ±0.1	+1.0 ±0.6	+0.2 ±0.2	-0.2 ±0.1	+4.0 ±1.2	-2.1 ±0.9

TABLE 12

sc x v car - 40°C. - Third Instar; 96 to 106 Hours -

Group	Size	Obser.	ps	as	an	pv	oc	or
"a"	152	1180	0.3 ±0.2	1.2 ±0.3	0.5 ±0.2	0.1 ±0.1	3.5 ±0.5	4.3 ±0.6
Control	158	1800	0.4 ±0.1	1.7 ±0.3	0.3 ±0.1	0.4 ±0.1	2.8 ±0.4	5.1 ±0.5
Difference			-0.1 ±0.2	-0.5 ±0.4	+0.2 ±0.2	+0.3 ±0.1	+0.7 ±0.6	-0.8 ±0.8
½	179	1058	0	1.4 ±0.4	0.2 ±0.1	0.2 ±0.1	5.3 ±0.7	3.4 ±0.6
Control	158	1800	0.4 ±0.1	1.7 ±0.3	0.3 ±0.1	0.4 ±0.1	2.8 ±0.4	5.1 ±0.5
Difference			-0.4 ±0.1	-0.3 ±0.5	-0.1 ±0.1	-0.2 ±0.1	+2.5 ±0.8	-1.7 ±0.7
1.	194	1116	0.1 ±0.1	2.0 ±0.4	0.1 ±0.1	0.3 ±0.2	4.8 ±0.6	3.1 ±0.5
Control	194	3930	0.6 ±0.2	2.7 ±0.3	0.3 ±0.1	0.5 ±0.1	2.5 ±0.3	4.4 ±0.4
Difference			-0.5 ±0.2	-0.7 ±0.5	-0.2 ±0.1	-0.2 ±0.2	+2.3 ±0.7	-1.3 ±0.6
1½	148	879	0.3 ±0.2	2.4 ±0.5	1.2 ±0.4	0.1 ±0.1	7.2 ±0.8	5.9 ±0.8
Control	158	1800	0.4 ±0.1	1.7 ±0.3	0.3 ±0.1	0.4 ±0.1	2.8 ±0.4	5.1 ±0.5
Difference			-0.1 ±0.2	+0.7 ±0.6	+0.9 ±0.4	-0.3 ±0.2	+4.4 ±0.9	+0.8 ±0.9

(continued on next page)

TABLE 12 (cont.)

Group	Size	Obser.	ps	as	an	pv	oc	or
2	163	984	0.6 ±0.3	2.0 ±0.5	0	0	7.5 ±0.8	6.8 ±0.8
Control	158	1800	0.4 ±0.1	1.7 ±0.3	0.3 ±0.1	0.4 ±0.1	2.8 ±0.4	5.1 ±0.5
Difference			+0.2 ±0.3	+0.3 ±0.6	-0.3 ±0.1	-0.4 ±0.1	+4.7 ±0.9	-1.7 ±0.9
$2\frac{1}{2}$	156	1334	0.4 ±0.2	2.8 ±0.5	1.4 ±0.3	0.5 ±0.2	6.6 ±0.8	5.2 ±0.6
Control	145	3634	0.4 ±0.1	2.2 ±0.3	0.3 ±0.1	0.4 ±0.1	3.1 ±0.3	5.9 ±0.4
Difference			0	+0.6 ±0.6	+1.1 ±0.3	+0.1 ±0.2	+3.5 ±0.9	-0.7 ±0.7
3	108	1058	0.1 ±0.1	2.4 ±0.5	0.3 ±0.2	0.8 ±0.3	2.9 ±0.5	4.4 ±0.6
Control	141	1388	0.3 ±0.1	2.6 ±0.4	0.2 ±0.1	0.4 ±0.1	3.4 ±0.5	7.1 ±0.7
Difference			-0.2 ±0.1	-0.2 ±0.6	+0.1 ±0.2	+0.4 ±0.3	-0.5 ±0.7	-2.7 ±0.9

TABLE 13

sc x v car - 40°C. - Young Pupae, 120 to 130 Hours -

Group	Size	Obser.	ps	as	an	pv	oc	or
Control	141	1388	0.3 ±0.1	2.6 ±0.4	0.2 ±0.1	0.4 ±0.2	3.4 ±0.5	7.1 ±0.7
"a"	151	888	0	1.4 ±0.4	0.1 ±0.1	0.9 ±0.3	6.4 ±0.8	6.9 ±0.9
Difference			-0.3 ±0.1	-1.2 ±0.6	-0.1 ±0.1	+0.5 ±0.4	+3.0 ±0.9	-0.2 ±1.1
1	134	1260	0.2 ±0.1	2.3 ±0.4	0.5 ±0.2	0.4 ±0.2	6.9 ±0.7	4.8 ±0.6
Difference			-0.1 ±0.1	-0.3 ±0.6	+0.3 ±0.2	0	+3.5 ±0.9	-2.3 ±0.9
2	164	1616	0.1 ±0.1	2.4 ±0.4	0.4 ±0.2	0.4 ±0.2	6.4 ±0.6	5.2 ±0.6
Difference			-0.2 ±0.1	-0.2 ±0.6	+0.2 ±0.2	0	+3.0 ±0.8	-1.9 ±0.9
3	114	1010	0.2 ±0.1	1.5 ±0.4	0.3 ±0.2	1.0 ±0.3	7.7 ±0.9	5.8 ±0.7
Difference			-0.1 ±0.1	-1.1 ±0.6	+0.1 ±0.2	+0.6 ±0.4	+4.3 ±1.0	-1.3 ±1.0
All	140	4774	0.1 ±0.05	2.0 ±0.2	0.3 ±0.1	0.6 ±0.1	6.8 ±0.4	*5.2 ±0.3
Difference			-0.2 ±0.1	-0.6 ±0.4	+0.1 ±0.1	+0.2 ±0.2	+3.4 ±0.7	-1.9 ±0.8

(* Sum of 1, 2, and 3, only.)

of the exposure in hours ("group"), the mean culture size in the series, the number of male half-fly observations, and for each bristle the percentile frequency of bristles present with the standard error of the frequency. For each exposure group the appropriate control data are given, and beneath them the difference between the experimental and control frequencies with its standard error.

a. The embryonic period.

Table 8 contains the data which show the effects of 40°C. on the bristle frequencies in sc males when the exposure is made during the embryonic life of the fly. The effect of the high temperature is extremely lethal during this period. Even in the "a" exposure series the culture size was small, and in the 3 hour series only an occasional adult developed. The lethal effect was probably at the time of exposure, and upon the embryo. In the 3 hour series, in cultures which developed no flies, no larvae were observed and the eggs were clearly visible in the food for several days.

The data of the "a", 1, and 2 hour exposure groups did not differ between themselves, and are therefore presented together. No contemporary controls of similar size were available. The difference in size probably accounts for the difference in the or frequency in the two groups of data. The increase in the oc frequency, however, is certainly significant, and the increase in an frequency is probably significant.

b. The first instar.

The data showing the effects of 40°C. during the first instar of larval life are those of Table 9. The high temperature was lethal to some of the larvae, expecially in the longer exposures. The data show that the main effect of the temperature on the bristles was an increase in the oc and or frequencies, especially the oc frequency, at nearly all exposures. The longer exposures appear also to have decreased the frequency of the pv bristle.

c. The second instar.

In Table 10 are the data from the series exposed during the first half of the second instar. The high temperature did not appear to be lethal during this period. The data show that the temperature increased the frequencies of the oc and or bristles and decreased the frequency of the pv bristle.

d. The first half of the third instar.

The data of Table 11 show the effects of high temperature during the first half of the third instar. The temperature effects were not noticeably lethal during this period. They are evident, however, in increases in the an and or frequencies. The last begins with the one hour exposure group and continues through the higher exposures. The changes in the ps and as frequencies, while nearly all in one direction, are probably not significant.

e. The second half of the third instar.

The Table 12 data show the effects of 40°C. during the last half of the third instar - the only period in which viable temperatures, according to Child, influence the bristle frequencies. The 3 hour exposure during this period was considerably more lethal than any of the shorter exposures. The exposure was repeated twice on a smaller scale with similar results. The data indicate a clear increase in the oc frequency for most of the exposures. Its return to the control level following the 3 hour exposure was born out in the smaller later tests. The changes in the other bristle frequencies are irregular, but indicate probable decreases in the pv and or frequencies.

f. The early pupal period.

The data in Table 13 are from flies which were exposed to 40°C. during what was the early pupal period for the majority of the individuals. There were a few larvae present, too. The 3 hour exposure in this period was more lethal than the shorter exposures. The significant temperature effects appear to have been on the oc and or bristles. The oc frequency is above the controls in each of the four exposure groups. The or frequency was unaffected by the "a" exposure, but was below the control frequency in the longer exposures, singly and combined.

g. Summary

Table 14 is a summary of the significant temperature effects on bristle frequencies demonstrated in the data of Tables 8 through 13. Each entry is the mean"coefficient

TABLE 14

sc x v car - Summary of 40°C. Series -

Developmental Period	ps	as	an	pv	oc	or
0 to 10 hours	0	0	+3.0	0	+2.32	0
20 to 30 hours	0	0	0	-0.55	+0.64	+0.44
48 to 50 hours	0	0	0	-0.74	+1.36	+0.63
72 to 82 hours	0	0	+3.0	-0.71	+0.89	-0.32
96 to 106 hours	0	0	0	-0.39	+0.89	-0.13
120 to 130 hours	0	0	0	0	+1.00	-0.27

of change" in bristle frequency in the experimental flies when compared to their respective controls. (See page 44). The mean coefficient of change is the average of the sum of the coefficients from the exposure groups of a given period, beginning with the shortest exposure to show a significant and including all the exposures longer than that one. The use of the mean coefficient of change is justified by the data of the above tables, which show that, generally speaking, a temperature effect upon a given bristle, once it has been established, is not consistently increased by longer exposures. An entry of 0 in this table indicates that there was no significant frequency change in that instance.

The data of Table 14 show the following general effects of exposures to 40°C. during the indicated developmental periods. The ps and as frequencies were not affected during any period of development. The an frequency increased in embryonic and early third instar periods to approximately four times the control frequency. The pv frequency decreased during the larval periods to one-half or one-quarter of the control frequency. The oc frequency increased in all the periods to approximately twice the control frequency. The or frequency increased in the first and second instars to approximately one and one-half times the control frequency; and decreased to approximately three-quarters of the control frequency during the third instar and young larval periods.

h. Discussion.

The data indicate clearly that the exposures to 40°C. have effected changes in the bristle frequencies of the sc males. We may now ask if the effect was on all or only a part of the population in each case, and if the bristles showed tendencies for covariance. The first of these questions may be answered by examination of the left-right correlation coefficients. These were calculated for each pair of bristles in each of the developmental periods. In no case did any coefficient appear to be different from its corresponding control. It seems probable, therefore, that the temperature effect was on the whole population. The question of covariance is normally answered by two-by-two correlation coefficients. They were not calculated in this experiment (see sc x sc experiment below). The data themselves, however, suggest that the bristles behave independently in this experiment, since, on the whole, the bristles do not respond alike to temperature treatment.

i. Conclusions.

We may make the following general conclusions from the above data:

(1) The TEP for sc at 40°C. extends throughout the developmental period for certain of the bristles. It varies in duration and position for the several bristles.

(2) The effect of 40°C. is not the same on all bristles. It increases the frequencies of some of them, and decreases the frequencies of others. Some bristles appear to be unaffected.

(3) The effect of 40°C. may not be in the same direction for a given bristle at different periods in development; but instead it may increase the frequency at one period and decrease it at another.

(4) The effect of 40°C., once established, is generally not changed by increasing the length of the exposure.

3. The data from sc x sc.

The 40°C. experiment with the mating sc females x sc males was done in the school year of 1936-1937 on the same general plan as the sc x v car experiment already discussed. The use of Moldex began with the second series in the second instar (see below) and was continued through the third instar and young pupae series.

The data of the males are given in Tables 15 through 21, in a manner similar to that used in the data tables of the sc x v car experiment. The number of observations in each exposure series was less than in the sc x v car experiment, and fewer series were completed. It was observed that the temperature effect was independent of the length of the exposure period, once it had begun. Therefore, in order to lower the standard deviations, the several exposure series have been grouped for comparison to their controls.

a. The embryonic period.

Table 15 contains the data from flies which were exposed to 40°C. during their embryonic developmental stage. More than one hour of 40°C. proved to be fatal to all but a very few of the embryos. The data show that one hour was sufficient to produce increases in the an and oc frequencies. The apparent increase in the or frequency is probably due to the considerably smaller mean size of the experimental cultures.

TABLE 15.

sc x sc - 40°C. - Embryo, 0 to 10 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
1	67	720	0.1 ±0.1	1.0 ±0.4	2.5 ±0.6	1.5 ±0.5	4.7 ±0.8	13.5 ±1.3
Control	110	1360	0.1 ±0.1	1.1 ±0.3	0.8 ±0.2	0.9 ±0.3	2.5 ±0.4	9.9 ±0.8
Difference			0	-0.1 ±0.5	+1.7 ±0.6	+0.6 ±0.6	+2.2 ±0.9	+3.6 ±1.5

TABLE 16.

sc x sc - 40°C. - First Instar, 20 to 30 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
1;2;3	137	1792	0	0.7 ±0.2	1.5 ±0.3	1.0 ±0.2	8.0 ±0.6	20.9 ±1.0
Control	125	1916	0	0.4 ±0.1	0.6 ±0.2	0.6 ±0.2	3.9 ±0.4	17.0 ±0.9
Difference			0	+0.3 ±0.2	+0.9 ±0.4	+0.4 ±0.3	+4.1 ±0.7	+3.9 ±1.3
2;3	97	1430	0	0.4 ±0.2	1.5 ±9.3	0.8 ±0.2	4.7 ±0.6	23.4 ±1.1
Control	110	2096	0	0.3 ±0.1	0.5 ±0.2	0.8 ±0.2	2.1 ±0.3	13.3 ±0.7
Difference			0	+0.1 +0.2	+1.0 +0.4	0	+2.6 +0.7	+10.1 +1.3

TABLE 17

sc x sc - 40°C. - Second Instar, 48 to 58 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
3	91	684	0.3 ±0.2	0.3 ±0.2	0.4 ±0.3	0.3 ±0.2	4.1 ±0.8	16.1 ±1.4
Control	110	2096	0	0.3 ±0.1	0.5 ±0.2	0.8 ±0.2	2.1 ±0.8	13.3 ±1.6
Difference			±0.3 ±0.2	0	-0.1 ±0.4	-0.5 ±0.3	±2.0 ±0.8	±2.8 ±1.6
2;3	108	1196	0	0.4 ±0.2	1.0 ±0.3	0.2 ±0.1	6.3 ±0.7	15.0 ±1.0
Control	108	3538	0	0.1 ±0.05	0.8 ±0.2	0.7 ±0.2	4.2 ±0.3	13.5 ±0.6
Difference			0	±0.3 ±0.2	±0.2 ±0.4	-0.5 ±0.2	±2.1 ±0.8	± 1.5 ±1.2

TABLE 18

sc x sc - 40°C. - Third Instar, 72 tO 82 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
2;3	163	2700	0.3 ±0.1	1.6 ±0.2	4.8 ±0.6	1.3 ±0.2	5.74 ±0.45	11.9 ±0.6
Control	155	7550	0.1 ±0.04	1.0 ±0.1	1.0 ±0.1	1.1 ±0.1	2.78 ±0.19	7.5 ±0.3
Difference			±0.2 ±0.1	±0.6 ±0.2	±3.8 ±0.4	±0.2 ±0.2	±3.0 ±0.5	±4.4 ±0.7

TABLE 19.

sc x sc - 40°C. - Third Instar, 96 to 106 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
2;3	150±	1126	0.3 ±0.2	2.2 ±0.4	3.9 ±0.6	1.2 ±0.3	9.7 ±0.9	6.0 ±0.7
Control	150	2932	0.3 ±0.1	1.6 ±0.2	1.5 ±0.2	0.5 ±0.1	5.6 ±0.4	5.9 ±0.5
Difference			0	+0.6 ±0.4	+2.4 ±0.6	+0.7 ±0.3	+4.1 ±1.0	+0.1 ±0.9

TABLE 20.

sc x sc - 40°C. - Young Pupae, 120 to 130 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
1;2;3	250±	1324	0	0.8 ±0.3	1.3 ±0.3	0.6 ±0.2	4.5 ±0.6	5.1 ±0.6
Control	240	7282	-0.04 ±0.02	0.6 ±0.1	0.9 ±0.1	0.4 ±0.1	5.0 ±0.3	5.1 ±0.3
Difference			-0.04 ±0.02	+0.2 ±0.3	+0.4 ±0.3	+0.2 ±0.2	-0.5 ±0.7	0

TABLE 21.

sc x sc - 40°C. - Older Pupae, 144 to 154 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
1;2;3	200±	1518	0	0.7 ±0.2	0.5 ±0.2	0.6 ±0.2	6.5 ±0.6	7.7 ±0.7
Control	199	4380	0.09 ±0.05	0.5 ±0.1	1.2 ±0.2	0.3 ±0.1	5.5 ±0.3	6.1 ±0.4
Difference			-0.09 ±0.05	+0.2 ±0.2	-0.7 ±0.3	+0.3 ±0.2	+1.0 ±0.7	+1.6 ±0.8

b. The first instar.

Data from flies exposed during the first part of the first instar are those in Table 16. The two sections of the table represent two series carried out several months apart. The data indicate increases in the an, oc, and or bristles.

c. The second instar.

The data from flies exposed during the second instar are presented in Table 17. The "3" hour group was raised on non-Moldex food; the "2;3" hour group was raised on Moldex food several months later, and at a slightly higher control temperature. The Moldex does not appear to have influenced the effect of the exposure to 40°C. on the bristle frequencies. The data indicate an increase in the oc and a decrease in the pv frequencies. When the data of the two tables are summed, the increase in the or is large enough to indicate probable significance (twice its standard error).

d. The first half of the third instar.

The data from flies exposed to the high temperature during the first half of their third larval instar of development are those of Table 18. They indicate increases in the frequencies of the an, oc, and or bristles. The slight increase in the other frequencies is probably not significant.

e. The second half of the third instar.

Table 19 contains the data from flies exposed to the high temperature during the second half of the third

instar. Many of the individuals died in the pupal stage. The culture size was estimated on the basis of a few rough counts of dead pupae, along with the fly counts. The data indicate increases in the an and oc bristle frequencies.

f. The early pupal period.

Because of the crowded conditions of cultures used in series testing the effect of temperature during the pupal stage, there was a considerable variation in the time of pupation. Some stragglers had failed to pupate even after 144 hours at 25°C. In addition, the mortality was very high among the pupae, many of which progressed to the imago stage but lacked the strength to emerge. Those that did emerge in the older pupae series were phenotypically abnormal to the extreme, and had very few hairs and bristles. They were classed for bristles by the presence or absence of the trichopore, out of which the bristle normally grows. Many pupae were on the sides of the culture; others were on the paper in the food. Between these two positions there was a difference of at least 1°C. For all of these reasons, the data from the pupae series which are given in Tables 20 and 21 are not as satisfactory as the larval data. They do not, however, indicate any strong effect of the temperature treatment on the bristles.

g. Summary.

The data of Tables 15 through 21 are summarized in Table 22, where each entry is the coefficient of change in bristle frequency. Table 22 indicates the following general facts about the response of the several bristles to

TABLE 22.

sc x sc - Summary of 40°C. Series -

Developmental Period	ps	as	an	pv	oc	or
0 to 10 hours	0	0	+2.1	0	+0.88	0
20 to 30 hours	0	0	+1.5	0	+1.15	+0.50
48 to 58 hours	0	0	0	-0.67	+0.73	+0.18
72 to 82 hours	0	0	+3.8	0	+1.08	+0.59
96 to 106 hours	0	0	+1.6	0	+0.73	0
120 to 130 hours	0	0	0	0	0	0
144 to 154 hours	0	0	0	0	0	0

the temperature treatment. The ps and as bristles were unaffected throughout development. The an frequency increased in all periods of egg-larval life excepting the second instar. The pv frequency decreased in the second instar but was unaffected during the other periods. The oc bristles increased in the first and second instars, and in the first half of the third instar. None of the bristles appear to have been affected during the pupal periods.

h. Discussion

To determine whether the temperature effects on sc males from sc x sc are on part or all of the population, the left-right correlation coefficients were calculated for each pair of bristles in each developmental period. In the embryo and first instar series, there was no indication of correlation in any of the bristle pairs. But in the older larvae, an increase in bristle frequency was accompanied by a small but probably significant increase in left-right correlation. In the control flies these correlations ranged from +0.02 to +0.08 with t values of 2 or more for only the larger coefficients. In the treated flies the coefficients ranged from +0.10 to+0.20 with t values of 3 to 5. This indicates that in these older series some of the larvae were probably unaffected by the exposure to high temperature. It is quite probable that these larvae were able to escape the extreme temperature by crawling into the paper in the middle of the food. The temperature was known to be a degree lower there than in the food, due probably to a higher evaporation of moisture from the paper.

To determine the possibility of covariance among the affected bristles in sc males from sc x sc, the two-by-two correlation coefficients were calculated for each of the possible combinations of the six bristles considered. These coefficients proved to be practically identical in experimental and control series, and in no cases were they large enough to suggest a tendency for covariance among any of the bristles.

i. Comparison with sc x v car.

The summarized data of sc x sc in Table 22 (page 86) are comparable to the summarized data of sc x v car in Table 14 (page 76). It is apparent at once that the outstanding facts of similarity in the temperature effects on the two matings are the absence of appreciable effects upon the ps and as bristles and the increase in oc frequency through practically all of development. The differences between the an and pv fequency changes are largely matters of duration of the sensitive period (TEP). The an bristle displays a considerably wider sensitive period in the sc x sc mating, while the pv bristle has a broader sensitive period in the sc x v car mating. The difference in the or frequency changes in the two matings involves both a difference in duration of the TEP and a difference in direction of bristle frequency change. In sc x sc, the change in frequency is an increase through a shorter portion of development. In sc x v car, the change is first an increase, then a decrease in frequency, over a considerably longer period.

j. Conclusions.

We may ~~make~~ draw the following general conclusions from the above considerations:

(1) The data of sc x sc support those of sc x v car in establishing a TEP for sc which extends for some bristles probably throughout the developmental period of the bristles, and differs in one way or another from bristle to bristle.

(2) They indicate, in addition, that the duration of the TEP and the nature of the effect produced vary between selected stocks.

4. Summary and conclusions from the 40°C. experiments.

(1) Data are presented which show the effects of a graded series of exposures to 40°C. during six developmental periods, covering practically the entire bristle development period, on the frequencies of certain of the bristles in sc males from the matings sc females x v car males and sc females x sc males.

(2) The ps and as bristles are unaffected in both matings.

(3) The an frequency increases to from 2 to 4 times the size of the control frequency, in both matings, through a part of the developmental period. The TEP is considerably greater in sc x sc than in sc x v car.

(4) The frequency of the pv bristles decreases in both matings to from one-quarter to three-quarters the size of the control frequency, through a part of the developmental period. The TEP is much broader in sc x v car than in sc x sc.

(5) The oc bristles increase in frequency to approximately twice the size of the control frequency in both matings, through all of egg-larval development and into pupal development in the sc x v car mating.

(6) The or frequency increases in sc x v car in the first and second instars to approximately 1½ times the size of the control frequency, and decreases to approximately three-quarters of the size of the control frequency in the third instar and early pupal periods. In sc x sc the or frequency increases in the first, second, and early third instar periods to approximately 1½ times the size of the control frequency and is unaffected in other developmental periods.

(7) The TEP of sc males for nearly lethal exposures to 40°C. extends throughout the bristle developmental period for one or more bristles.

(8) The duration of the TEP, and the extent and direction of the change in bristle frequency varies more or less from bristle to bristle, from stock to stock, and from one developmental period to another.

(9) A change in bristle frequency effected by a given exposure to 40°C. within a given devlopmental period is generally not increased by increasing the length of the exposure period.

(10) A lack of correlation between any two of the bristles in the sc x sc experiment indicates that the 40°C. treatment affects the bristles independently of each other.

E. The 36°C. Experiments with sc Males.

1. Plan of experiments.

During the spring and fall ~~school terms~~ of 1937, two experiments with 36°C. were carried out, similar in plan and method to the above 40°C. experiments, but less extensive in the number of tests made. Rough experiments in the early months of 1935 had indicated that nearly lethal exposures to 36°C. produced very different effects on the bristle frequencies than did nearly lethal exposures to 40°C.

The main difference in method in the two temperature experiments was the use of much longer exposure periods in the 36°C. experiment, and hence the testing of much broader developmental periods. The entire first instar constituted one test period; the entire second instar constituted another; the third instar was divided into two test periods; and the early pupal period constittuted a fifth period. In addition, the embryonic period was tested in the sc x sc experiment.

2. The data from sc x v car.

The 36°C. sc x v car experiment was done in May, 1937. It was necessary to carry out the experiment at a control temperature between 25.5 and 26°C. The 26°C. control data are those of Table 7 previously discussed. The data from the sc males of the experimental lines are presented in Tables 23 through 27. These tables are set up in a manner similar to that used in presenting the 40°C. data.

TABLE 23.

sc x v car - 36°C. - First Instar, 24 to 53 Hours Development

Group	Size	Obser.	ps	as	an	pv	oc	or
16;22	171	1400	2.4 ±0.4	14.0 ±0.9	1.9 ±0.4	1.1 ±0.3	9.9 ±0.8	6.8 ±0.7
Control	196	1316	0.8 ±0.2	4.3 ±0.5	1.6 ±0.3	2.0 ±0.4	10.6 ±0.9	3.3 ±0.5
Difference			+1.6 ±0.4	+9.7 ±1.0	+0.3 ±0.5	-0.9 ±0.5	-0.7 ±1.2	+3.5 ±0.9

TABLE 24.

sc x v car - 36°C. - Second Instar, 44 to 82 Hours Development

Group	Size	Obser.	ps	as	an	pv	oc	or
24	211	1618	1.7 ±0.3	8.2 ±0.7	1.1 ±0.3	0.4 ±0.2	12.9 ±0.8	4.0 ±0.5
Control	242	1848	1.1 ±0.2	5.4 ±0.5	1.7 ±0.3	1.5 ±0.3	6.5 ±0.6	1.8 ±0.3
Difference			+0.6 ±0.4	+2.8 ±0.9	-0.6 ±0.4	-1.1 ±0.4	+6.4 ±1.0	+2.2 ±0.6
30	204	1280	1.5 ±0.3	10.5 ±0.9	1.9 ±0.4	0.6 ±0.2	22.5 ±1.2	6.2 ±0.7
Control	196	1316	0.8 ±0.2	4.3 ±0.5	1.6 ±0.3	2.0 ±0.4	10.6 ±0.9	3.3 ±0.5
Difference			+0.7 ±0.4	+6.2 ±1.0	+0.3 ±0.5	-1.4 ±0.4	+11.9 ±1.5	+2.9 ±0.9

TABLE 25.

sc x v car - 36°C. - Third Instar, 68 to 105 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
24	228	1322	7.3 ±0.7	12.9 ±0.9	2.6 ±0.4	0.5 ±0.2	10.4 ±0.9	6.4 ±0.7
Control	237	3234	0.9 ±0.2	5.6 ±0.4	2.4 ±0.3	1.6 ±0.2	10.6 ±0.5	2.7 ±0.3
Difference			+6.4 ±0.7	+7.3 ±1.0	+0.2 ±0.5	-1.1 ±0.3	-0.2 ±1.0	+3.7 ±0.8
30	204	1326	16.4 ±1.0	22.2 ±1.1	2.3 ±0.4	0.7 ±0.2	11.5 ±0.9	4.8 ±0.6
Control	196	1316	0.8 ±0.2	4.3 ±0.5	1.6 ±0.3	2.0 ±0.4	10.6 ±0.9	3.3 ±0.5
Difference			+15.6 ±1.0	+17.9 ±1.2	+0.7 ±0.5	-1.3 ±0.4	+0.9 ±1.3	+1.5 ±0.8

TABLE 26.

sc x v car - 36°C. - Third Instar, 90 to 128 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
24	277	1708	12.4 ±0.8	15.5 ±0.9	3.5 ±0.4	0.5 ±0.2	7.5 ±0.6	2.8 ±0.4
Control	278	1918	1.0 ±0.2	6.6 ±0.6	3.0 ±0.4	1.4 ±0.3	10.6 ±0.7	2.3 ±0.3
Difference			+11.4 ±0.8	+ 8.9 ±1.1	+0.5 ±0.6	-0.9 ±0.4	-3.1 ±0.9	+0.5 ±0.5
30	237	734	20.4 ±1.5	22.8 ±1.6	2.2 ±0.5	0.3 ±0.2	14.9 ±1.3	8.3 ±1.0
Control	237	3234	0.9 ±0.2	5.6 ±0.4	2.4 ±0.3	1.6 ±0.2	10.6 ±0.5	2.7 ±0.3
Difference			+19.3 ±1.5	+17.2 ±1.7	-0.2 ±0.6	-1.3 ±0.3	+4.3 ±1.4	+5.6 ±1.0

TABLE 27.

sc x v car - 36°C. - Young Pupae, 116 to 148 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
25	237±	832	2.0 ±0.5	4.7 ±0.7	1.8 ±0.5	0	6.2 ±0.8	6.6 ±0.9
Control	237	3234	0.9 ±0.2	5.6 ±0.4	2.4 ±0.3	1.6 ±0.2	10.6 ±0.5	2.7 ±0.3
Difference			+1.1 ±0.5	-0.9 ±0.8	-0.6 ±0.6	-1.6 ±0.2	-4.4 ±0.9	+3.9 ±1.3

a. The first instar.

Table 23 contains the data from flies exposed to 36°C. during the first instar. An earlier series with a 24 hour exposure to 36.5°C. failed to hatch many flies; but 16 and 22 hours of 36°C. were not markedly lethal. There were no differences in the bristle frequencies of the two series, and the data are presented here together. It can be seen that there was a substantial increase in ps, as, and or bristles, and a decrease in the pv bristles.

b. The second instar.

The data of Table 24 are from flies exposed during the second instar. The exposure to 36°C. appears to have reduced the pv frequency. The difference between the 24 and 30 hour exposures is possibly reflected in a somewhat larger increase in the ps and as frequencies in the 30 hour group.

c. The first half of the third instar.

The data of Table 25 are from flies exposed to 36°C. during the first half of the third instar. The ps and as frequencies are very much larger in the experimental series, and considerably more so in the 30 hour exposure than in the 24 hour group. The pv frequency is smaller in the experimental series. The or frequency is larger.

d. The second half of the third instar.

The data of Table 26 are from flies heated during the last half of the third instar and in early pupal life.

Many larvae had pupated at the time exposure was begun. None appeared to pupate during the exposure period; but nearly all the larvae pupated within a few hours after removal from the high temperature. Only a few flies failed to emerge from the pupae cases in the 24 hour group; but a majority failed to emerge in the 30 hour group. The data are not unlike those of the first half of the instar in Table 25, excepting a curious behavior of the oc frequency. In the 24 hour exposure group, the oc frequency is below the control frequency by more than three times the standard error of difference. In the 30 hour groups, it is above the control frequency by approximately the same amount. A deviation as great as either of these between two means would be expected to occur by chance alone in the order of once in 300 trials. It is probable, therefore, that this is a true temperature effect, and not a random variation.

e. The early pupal period.

The data of Table 27 are from flies exposed to 36°C. during the early pupal period. Only a few individuals were still larvae at the time of exposure. Most of the flies failed to emerge from the pupae cases. As in earlier pupae series, those which did emerge were markedly abnormal phenotypically. Bristle classification in many cases was by means of the trichopore. The data indicate an increase in the or frequency and a decrease in the oc and pv frequencies. The small ps frequency increase was probably due to an effect on the few larvae present (discussed below).

f. Summary.

The data of Tables 23 through 27 are summarized in Table 28. As in previous summaries, each entry is the coefficient of change in bristle frequency. The data of this table show the following general observational facts. The ps frequency increased throughout larval development, and very markedly in the third instar. The effect of 30 hours was greater than the effect of 24 hours in at least the two third instar periods. The as frequency likewise increased throughout larval development, and probably somewhat more in the third instar than in the first two instars. The effect of 30 hours was considerabley greater than the effect of 24 hours. The an bristle was unaffected in all developmental periods. The pv frequency was reduced to from one-half to one-quarter the size of the control frequency in every developmental period. The oc frequency increased in the second instar to twice the size of the control frequency. In the late third instar period it was reduced by the 24 hour exposure and increased by the 30 hour exposure, neither change being large, but each being statistically significant. In the early pupal period the oc frequency was reduced to six-tenths of the control frequency. The or frequency increased in all developmental periods, generally to about twice the size of the control frequency. There does not appear to have been any significant difference between the effects of 24 hours and the effects of 30 hours upon the frequencies of the pv and or bristles, and upon the frequency of the oc bristles in the second instar.

TABLE 28

sc x v car - Summary of 36°C. Series -

Period	Exposure	ps	as	an	pv	oc	or
24 to 53	16;22	+2.0	+2.3	0	-0.45	0	+1.1
48 to 82	24	+0.5	+0.5	0	-0.73	+1.0	+1.2
	30	+0.9	+1.4	0	-0.70	+1.1	+0.9
68 to 105	24	+7.1	+1.3	0	-0.69	0	+1.4
	30	+19.5	+4.2	0	-0.65	0	+0.46
90 to 128	24	+11.4	+1.4	0	-0.64	-0.29	+0.22
	30	+21.4	+3.1	0	-0.81	+0.41	+2.1
116 to 148	25	0	0	0	-1.0	-0.40	+1.4

g. Discussion.

To determine if the temperature effect was on part or all of the population in a culture, left-right correlation coefficients have been calculated and are presented in Table 29. Included under each coefficient is its t value.

It can be seen that in the control series of this experiment there was a small positive correlation in all but the oc bristles. In earlier series with this mating the an correlation was generally less than here, and the oc frequency was generally positive, between 0.02 and 0.08.

In the first instar series the coefficients do not appear to have changed significantly from the controls. Beginning with the second instar there appears to have been an increase in several of the coefficients. This is especially notable in the ps and as coefficients in the two third instar periods, which was also the time of greatest increase in the ps frequency. It seems probable that the temperature effect was not the same on the whole population in these older larvae, and that in the cases of the ps and as bristles more larvae were affected in the 30 hour exposures than in the 24 hour exposures. The possibility must also be recognized, however, that the difference between the two exposures may have resulted from the extra six hours of exposure upon the same larvae, in the 30 hour group. These data are not critical for this interpretation.

The relatively very large increase in ps frequency in the third instar, with accompanying large left-right correlation coefficients, suggests for this bristle

TABLE 29.

Left-right Correlation Coefficients in 36°C. sc x v car -

Period	Exposure	ps	as	an	pv	oc	or
- Control -		+0.06 2.8	+0.13 6.3	+0.10 5.1	+0.04 2.0	+0.01 0.5	+0.04 1.7
24 to 53	16;22	+0.04 0.9	+0.05 1.3	-0.02 0.5	0	+0.02 0.5	+0.09 2.2
44 to 82	24	+0.06 1.5	+0.17 4.9	0	0	+0.08 2.4	+0.06 1.5
	30	0	+0.22 5.5	0	0	+0.11 2.7	+0.12 3.0
68 to 105	24	+0.30 7.8	+0.23 6.0	+0.09 2.4	0	+0.14 3.6	+0.19 4.8
	30	+0.29 7.5	+0.27 7.0	+0.11 2.9	0	+0.06 1.7	+0.11 2.9
90 to 128	24	+0.53 15.0	+0.32 9.2	+0.14 4.2	0	+0.09 2.5	+0.13 3.7
	30	+0.53 10.0	+0.19 3.5	0	0	+0.11 2.0	+0.16 3.0
116 to 148	25	+0.46 9.4	+0.11 2.3	+0.12 2.4	0	+0.14 2.8	+0.20 4.1

an especially sensitive period which is more or less similar in position in development to Child's TEP at viable temperatures. These data are not suitable for establishing this as a fact.

In the case of the relatively large left-right correlation coefficient of the ps bristles in the 116 to 148 hour period, there was only a small increase in ps frequency. This shows that the temperature effect was probably limited to a very few individuals. Since there were a few larvae present at the beginning of the exposure period, it is probable that they, rather than some of the many pupae, were affected. Accordingly, this increase has not been interpreted as indicating a sensitive period in the pupal stage.

Correlation coefficients were also calculated for the two-by-two comparisons of the six bristles in this experiment. There was no consistent correlation between any of the bristles in any of the developmental periods. This indicates a probable independence of the response of each of the several bristles to the temperature treatment.

h. Comparison with 40°C. sc x v car.

The summarized 36°C. sc x v car data of Table 28 may be compared with the similarly summarized 40°C. sc x v car data of Table 14 (page 76). The most notable difference is in the effect upon the ps and as bristles. The 40°C. treatment did not affect these bristles; the 36°C. treatment increased their frequencies more than any of the others. The an bristles increased in frequency in two periods in the 40°C. experiment, but were unaffected in the 36°C.

experiment. The pv frequency was affected over a somewhat broader period in the 36°C. experiment. The oc frequency was affected over a much broader period in the 40°C. experiment. The or frequency, which increased in every period in the 36°C. experiment, showed a change in direction of response in the 40°C. experiment, where it increased in the first two instars and decreased in the third instar and early pupal period. Thus, every bristle responded more or less differently to the two temperatures. That this is due in the main to the difference in temperature treatment and not to the Moldex food of the 36°C. experiment is evidenced by the fact that the preliminary 35 to 36°C. experiment previous to the use of Moldex showed essentially the same phenomena, especially in regard to the ps and as bristles.

3. The data from sc x sc.

In February, October, and November of 1937 a 36°C. experiment was carried out with the mating sc females x sc males, using practically the same methods as in the 36°C. sc x v car experiment. The pure sc individuals could not withstand as long an exposure to the high temperature as could the hybrids. Accordingly, in this experiment the exposures were, for the most part, 12 to 24 hours in lenght.

In the February part of the experiment, tests were made on the embryonic and early larval developmental periods. Flies were allowed to oviposit for 6 to 8 hours in a culture, and after their removal, the culture was immediately exposed to 36°C. The embryos proved incapable of withstanding 6 hours of this temperature. Twenty such cultures gave practically

no adults; nor were larvae visible in the cultures which did not hatch flies. Another series of cultures in the time period of 15 to 34 hours of development did not appear to be adversely affected by 6 or 12 hours of 36°C., but could not take 24 hours. While many of the younger individuals in these cultures were undoubtedly still in the eggs during at least part of the exposure period, most of them had probably completed embryonic differentiation, according to the data of Poulson (1935). This period is therefore considered practically as a test of young larvae rather than of embryos.

The rest of the data of this experiment is from the series run in October and November of 1937.

The data are presented in Tables 30 through 35.

a. The first instar.

The data of the early first instar are given in Table 30. Because there was no difference in bristle frequencies in the 6 and 12 hour exposures the two series have been summed in this table. The data show a small increase in the frequencies of the an, oc, and or bristles.

The data from larvae further along in the first instar, are given in Table 31. There were apparent differences in the 12 and 24 hour exposure groups. Together they show increases in the as, an, oc, and or frequencies.

b. The second instar.

The data from the second instar series are those of Table 32, in which again the 12 and 24 hour groups are presented together because of lack of difference between them.

TABLE 30.

sc x sc - 36°C. - First Instar, 15 to 34 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
6;12	153	2388	0.04 ±0.02	0.7 ±0.2	1.5 ±0.3	0.8 ±0.2	6.9 ±0.5	16.7 ±0.8
Control	149	2216	0.09 ±0.06	0.4 ±0.1	0.6 ±0.1	0.7 ±0.2	5.5 ±0.5	13.2 ±0.7
Difference			-0.05 ±0.06	+0.3 ±0.2	+0.9 ±0.3	+0.1 ±0.2	+1.4 ±0.7	+3.5 ±1.1

TABLE 31.

sc x sc - 36°C. - First Instar, 24 to 57 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
12;24	117	1298	0.1 ±0.1	1.2 ±0.3	1.7 ±0.4	0.4 ±0.2	5.0 ±0.6	17.0 ±1.0
Control	162	1812	0.1 ±0.1	0.2 ±0.1	0.6 ±0.2	0.6 ±0.2	3.1 ±0.4	8.3 ±0.6
Difference			0	+1.0 ±0.3	+1.1 ±0.4	-0.2 ±0.3	+1.9 ±0.7	+8.7 ±1.2

TABLE 32.

sc x sc - 36°C. - Second Instar, 48 to 80 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
12;24	117	1424	0	0.35 ±0.17	1.5 ±0.3	0.2 ±0.1	4.4 ±0.5	7.9 ±0.7
Control	146	1434	0	0.07 ±0.07	0.7 ±0.2	0.4 ±0.2	2.2 ±0.4	8.9 ±0.8
Difference				+0.28 ±0.17	+0.8 ±0.4	-0.2 ±0.2	+2.2 ±0.6	-1.0 ±1.1

TABLE 33 A.

sc x sc - 36°C. - Third Instar, 72 to 92 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
12	214	1220	1.2 ±0.3	5.8 ±0.7	0.5 ±0.2	0.5 ±0.2	8.3 ±0.8	6.6 ±0.7
Control	186	1538	0.1 ±0.1	0.6 ±0.2	1.8 ±0.3	0.5 ±0.2	6.5 ±0.6	12.6 ±0.9
Difference			+1.1 ±0.3	+5.2 ±0.7	-1.3 ±0.4	0	+1.8 ±1.0	-6.0 ±1.2

TABLE 33 B.

sc x sc - 36°C. - Third Instar, 72 to 104 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
24	191	1164	7.8 ±0.8	16.8 ±1.1	0.6 ±0.2	0.4 ±0.2	4.9 ±0.6	5.5 ±0.7
Control	186	1538	0.1 ±0.1	0.6 ±0.2	1.8 ±0.3	0.5 ±0.2	6.5 ±0.6	12.6 ±0.9
Difference			+7.7 ±0.8	+16.2 ±1.1	-1.2 ±0.4	-0.1 ±0.3	-1.6 ±0.9	-7.1 ±1.2

TABLE 34.

sc x sc - 36°C. - Third Instar, 96 to 116 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
12	200±	902	0.2 ±0.1	4.0 ±0.7	4.0 ±0.7	0.1 ±0.1	3.7 ±0.6	4.9 ±0.7
Control	195	3430	0.1 ±0.02	0.3 ±0.1	0.7 ±0.1	0.5 ±0.1	2.9 ±0.3	8.1 ±0.5
Difference			+0.1 ±0.1	+3.7 ±0.7	+3.7 ±0.7	-0.4 ±0.1	+0.8 ±0.7	-3.2 ±0.9

TABLE 35.

sc x sc - 36°C. - Young Pupae, 120 to 152 Hours Development -

Group	Size	Obser.	ps	as	an	pv	oc	or
12	200±	676	0.1 ±0.1	0.3 ±0.2	0.9 ±0.4	0.3 ±0.2	3.3 ±0.7	6.1 ±0.9
Control	195	3430	0.1 ±0.02	0.3 ±0.1	0.7 ±0.1	0.5 ±0.1	2.9 ±0.3	8.1 ±0.5
Difference			0	0	+0.2 ±0.4	-0.2 ±0.2	+0.4 ±0.7	-2.0 ±1.1

The high temperature increased the frequencies of as, an, and oc bristles. The changes in the as and an frequencies are on the border line of statistical significance.

c. The first half of the third instar.

The data from the first half of the third instar are given in Tables 33 A and 33 B. There was a marked difference in the temperature effect on the ps and as frequencies in the 12 and 24 hour exposures. These have therefore been given separately. The data show decreases also in the frequencies of the pv and or bristles, without any apparent difference between the two exposures. The 24 hour exposure produced much greater increases in the ps and as frequencies than did the 12 hour exposure. The 12 hours difference in time, it will be noted, was entirely at the upper time limit; i.e., the larvae were exposed 12 hours later in the third instar in the 24 hour exposure.

d. The second half of the third instar.

The data from the later third instar series are given in Table 34. The 24 hour exposure was almost entirely lethal, and the 12 hour exposure was lethal to many individuals. As in the earlier experiments, death appeared to come in the pupal stage. Some larvae had pupated at the time of the exposure; but most of them had not. The majority of the larvae appeared to pupate within a very few hours after removal from the high temperature. The data of the table indicate increases in the as and an frequencies, and decreases in the pv and or frequencies.

e. The early pupal period.

The data from the early pupal period are those of Table 35. The 24 hour exposure was highly lethal, and the 12 hour exposure was about 50% lethal. The flies that emerged were generally extremely abnormal phenotypically, as in previous pupal series. The data of the table indicate no significant bristle frequency changes.

f. Summary.

The data of Tables 30 through 35 are summarized in Table 36. As in previous summaries, each entry is the coefficient of change in bristle frequency. The following summary of effects can be made from this table. The ps frequency was affected only in the first of the third instar series, where it increased markedly, especially following the 24 hour exposure. The as frequency increased through all but the early part of larval life, and showed a much larger increase following the 24 hour exposure during the third instar. The an frequency increased in the first two instar periods; decreased in the first of the third instar series, and increased again in the late third instar. The pv frequency decreased in the late third instar. The oc frequency increased in the first two instars. And the or frequency increased in the first instar, and decreased in the third instar. None of the frequencies changed significantly in the early pupal period.

g. Discussion

As in the previous experiments, left-right correlation coefficients were calculated to determine whether

TABLE 36.

sc x sc - Summary of 36°C. Series

Period	Exposure	ps	as	an	pv	oc	or
15 to 34	6;12	0	0	+1.5	0	+0.3	+0.3
24 to 57	12;24	0	+5.0	+1.8	0	+0.6	+1.0
48 to 80	12;24	0	+4.0	+1.1	0	+1.0	0
72 to 92	12	+11	+8.7	-0.72	0	0	-0.48
72 to 104	24	+77	+27.0	-0.67	0	0	-0.56
96 to 116	12	0	+12.0	+5.3	-0.8	0	-0.40
120 to 140	12	0	0	0	0	0	0

the temperature effect was on all or only a part of the population in each period. There appeared to be differences between the control and experimental coefficients only in the ps and as bristles in the third instar series. In the case of the ps bristles, the only apparent left-right correlation was in the 24 hour exposure of the 72 to 104 hour period. The coefficient was +0.35 with a t value of 8. The correlation coefficient was approximately 0 in the control series. In the case of the as bristles, the control coefficient was +0.12 with a t value of 3.4. In the 12 to 24 hour exposure groups of the 72 to 104 hour period, and in the 12 hour exposure of the 96 to 116 hour period, the coefficients were +0.39, +0.40, and +0.54, respectively. The t values for these coefficients ranged from 9 to 11. From this it appears that although the temperature effect was more or less on the whole population with respect to pv and or bristles in these third instar series, it was on only a part of the population with respect to the ps and as bristles.

The marked differences between the 12 and 24 hour exposures in the 72 to 104 hour period with respect to their effects on the ps and as bristles is probably due to more larvae having been affected during the 24 hour exposure. As in the sc x v car experiment, the magnitude of the increase in these bristles in this period and in the as bristle in the following period suggest that there probably exists, well in the third instar, an especially sensitive period for these bristles, which may correspond to Child's TEP for all the

bristles at lower temperatures.

Two-by-two correlation coefficients were not calculated for the bristles in this experiment; but there is no reason to suppose that any more correlation existed here than in the 36°C. sc x v car experiment.

h. Comparison with 36°C. sc x v car.

A comparison may be made between the summarized data in Table 36 with the similarly summarized 36°C. data from sc x v car in Table 28 (page 99). The data differ to some extent for each of the six bristles and indicate the presence of effective modifiers of sc-affected bristles in the two stocks. The data are alike in indicating an especially sensitive period for as bristles, and to a lesser degree for as bristles, in the third instar.

i. Comparison with 40°C. sc x sc.

A comparison may be made between the summarized data of Table 36 and the similarly summarized 40°C. sc x sc data in Table 22 (page 86). The most notable difference between the two sets of data is in the response of the ps and as bristles, which was also the case in the comparison of the two similar sc x v car experiments. There appear also to be differences between the other bristles in these two sc x sc experiments.

Curiously enough, the response of the or frequency to 36°C. in the sc x sc mating resembles more its response to 40°. in the sc x v car mating than it does its response to 40°C. in the sc x sc mating. In both

the 36°C. sc x sc and the 40°C. sc x v car experiments there is a reversal in the direction of or bristle frequency change beginning in the third instar.

Since much of the 40°C. sc x sc experiment was done on Moldex food similar to that used in the 36°C. experiment, it seems probable that the differences between the two experiments reflect differences in bristle response to the two temperature treatments.

4. Summary and conclusions from the 36°C. experiments.

(1) Data are presented from sc males from the mating sc x v car and sc x sc which show the effects of nearly lethal exposures to 36°C. on the frequencies of six of the sc-affected bristles.

(2) Each of the bristles differs in some respect in its response to 36°C. in the sc x v car mating as compared to its response to 36°C. in the sc x sc mating.

(3) The ps frequency increases throughout larval life in sc x v car, and especially in the third instar. In sc x sc it increases only during the third instar.

(4) The as frequency increases practically throughout larval life in both matings, but relatively more in sc x sc. The increase is especially marked in the third instar in sc x sc.

(5) The an frequency is unaffected in sc x v car. It increases in the first, second, and late third instars, and decreases in the first half of the third instar, in sc x sc.

(6) The pv frequency decreases throughout larval and early pupal development in sc x v car. It decreases in the late third instar in sc x sc.

(7) The oc frequency increases in the second instar, decreases following 24 hours exposure and increases following 30 hours exposure in the late third instar, and decreases in the early pupal period in sc x v car. It increases in the first two instars in sc x sc.

(8) The or frequency increases throughout larval and early pupal development in sc x v car. It increases in the first

instar and decreases in the third instar in sc x sc.

(9) The effect of 30 hours exposure is greater than that of 24 hours on the ps and as frequencies in the third instar of sc x v car. The effect of 24 hours is greater than the effect of 12 hours on the ps and as frequencies in the third instar in sc x sc. These differences do not extend in general to the other affected bristles.

(10) The greater effect of exposures during the third instar upon the ps frequency in both matings, and upon the as frequency in sc x sc, suggests a period of higher sensitivity to the temperature treatment, possibly related to Child's TEP for all the bristles at lower temperatures.

(11) The lack of correlation between any two of the bristles in the sc x v car experiment indicates that the 36°C. treatment affected the several bristles independently of each other.

(12) The data confirm those from the 40°C. experiments in establishing a TEP for sc which extends for one bristle or another practically throughout the developmental period, as it relates to bristles.

(13) In one or both matings, each of the several frequencies responds differently to 36°C. than it does to 40°C., the difference being most pronounced in the ps, as, and oc bristles.

(14) The nature of the change in bristle frequency in sc males following a nearly lethal exposure to high temperature depends upon (a) the temperature treatment, (b) the bristle modifiers present in the sc stock, (c) the bristle frequency under consideration, and (d) the developmental period during which the treatment takes place.

F. Other Temperature Experiments with sc Males.

1. Effects of 40 and 36°C. on F_1 males from treated parents.

Bristle frequencies were determined in F_1 males from treated sc flies of the 40 and 36°C. experiments discussed above. The parent flies were from the longest one or two of the exposures in each developmental period tested in these high temperature experiments. Each developmental period was represented by from 2000 to 5000 observations. In no case did the bristle frequencies of these F_1 sc males deviate significantly and consistently from the respective frequencies of their cousin control F_1 raised under similar conditions of food, larval density, and temperature.

At the time of exposure to the high temperature, these F_1 individuals were probably primordial or developing germ cells in the gonads of the larvae or pupae which were being exposed to the high temperature. From the results it seems certain that the high temperature treatment which is able in some way to effect a change in adult bristle frequencies of sc larvae is not able to effect such a change during the germ cell stage.

2. Experiments with lower temperatures.

During the winter and spring school terms of 1936 I attempted a series of experiments designed to test sc male bristle frequency changes following a three hour exposure to one or another of a graded series of seven temperatures between 30 and 40°C. The exposures were made, in one experiment, during the first instar, and, in another experiment, during the second instar. The mating was sc females x v car males as in the 1934-1935 experiment. Unfortunately, it was not possible to secure environmental conditions constant enough to make the data suitable for detailed analysis. For that reason, the data are not presented here. It appeared to be probable, however, that such a short exposure to temperatures below approximately 39°C. during these two developmental periods does not effect significant changes in sc male bristle frequencies.

G. Temperature Effects on the Dominant Manifestation of sc.

1. Introduction.

The gene sc is normally classed as a recessive gene because of its marked effect on the bristles when in homozygous condition. At ordinary room temperatures it does not show an appreciable dominant effect on these bristles, with the exception of the coxal bristle on the third pair of legs. This last bristle shows a high frequency of absence in sc/non-sc females.

It was noted in the 1934-1935 40°C. sc x v car experiment reported above that sc/v car females do occasionally lack a bristle of those which were being tabulated in the sc males; and that this phenomenon seemed to occur more frequently in treated than in control females. The preliminary experiment with 36°C. in the early months of 1935 revealed a profound decrease in frequency in certain ~~of~~ bristles in sc/v car females. As a result, in all subsequent sc x v car experiments, at both 40 and 36°C., the bristle frequencies were tabulated from the females as well as from the males. The conditions and procedure of these experiments have been described in connection with the male data.

A 36°C. experiment was also performed with the synthetic wild stock derived from the sc and v car stocks, in order to determine the effects of temperature upon non-sc modifiers of bristle frequencies. The results make possible an estimate both of the probable dominant effect of the sc gene itself and of the probable effect of 36°C. upon the sc reaction.

2. The effects of 40°C. on sc/v car females.

Tabulation of sc/v car bristle data in the 40°C. experiment was limited to part of the 2½ and 3 hour exposure groups in the 96 to 106 hour developmental period, and to all of the exposures in the embryonic (0 to 10 hours) and early pupal (120 to 130 hours) periods. The data are given in Table 37 in a form similar to that used in the tables of male bristles. In this case, however, each entry represents the percentile frequency of bristles absent per half-fly. Since the usual hybrid fly displays all of these brisltes, the only measurable effect of temperature was a decrease in bristle frequency.

Because there were no apparent differences between the exposure groups with respect to bristle frequencies, the several exposures are presented together in each of the periods in this table. The data show a small but significant temperature effect upon the ps, as, pv, and oc frequencies in the 0 to 10 and 96 to 106 hour periods. In the 120 to 130 hour period, the effect upon the pv frequency was less marked and there does not appear to be any difference between control and experimental or frequencies. Although there are no data from the intermediate developmental periods, the impression sustained through those series was that there was a slightly larger frequency of absence, especially of the pv bristles, in the treated females. It is probable, therefore, that the temperature treatment produced a small effect upon heterozygous female bristle frequencies throughout the entire developmental period of the bristles.

TABLE 37.

sc/v car - 40°C. - Embryonic, Larval, Pupal Exposures -

Period	Exposure	Obser.	ps	as	an	pv	oc	or
Control		13,648	0	0.007 ±0.007	0.007 ±0.007	0.63 ±0.07	0.029 ±0.015	0
0 to 10	"a";1;2	1596	0.13 ±0.09	0.13 ±0.09	0	2.13 ±0.29	0.13 ±0.09	0
96 to 106	2½;3	1358	0.07 ±0.07	0.07 ±0.07	0	2.43 ±0.42	0.30 ±0.15	0
120 to 130	"a";1; 2;3	5782	0.09 ±0.04	0.07 ±0.03	0	1.33 ±0.15	0.03 ±0.02	0
- Total -		8736	0.092 ±0.032	0.080 ±0.030	0	1.65 ±0.14	0.092 ±0.032	0
Difference from Control			+0.092 ±0.032	+0.073 ±0.031	-0.007 ±0.007	+1.02 ±0.16	+0.063 ±0.035	0

3. The effects of 36°C. on sc/v car females.

Preliminary experiments in 1935 showed that exposures of one to two days to a temperature of 35 to 36°C. during larval development produced a marked decrease in bristle frequencies in sc/v car females. Accordingly, in the 1937 36°C. sc x v car experiment the heterozygous sc females were recorded for frequency of absence of bristles.

a. The data.

The data of sc/v car females from this experiment are given in Table 38, in a form similar to that used in Table 37. They show that the temperature treatment affected the ps, pv, and oc frequencies in the later larval and early pupal periods. The temperature effects are strongest on all bristles in the third instar and early pupal period exposures. There are differences between the several bristles, however, with respect to the time of maximum sensitivity. Thus, the ps and as frequencies appear to be most strongly affected by the 30 hour exposure during the 68 to 105 hour period; the an frequency change reaches a decided maximum in the 30 hour exposure during the 90 to 128 hour exposure; the pv frequency change is relatively very large in both exposures of the 90 to 128 hour period and the one exposure of the 116 to 148 hour period; the oc bristle is most affected by the 30 hour exposure of the 90 to 128 hour period and by the exposure in the pupal period; and the or frequency change is most prominent during the pupal period exposure. It appears, then, that in sc/v car females, the temperature effect upon the several bristles differs significantly from bristle to

TABLE 38.

sc/v car - 36°C. - Larval and Young Pupal Exposures

Period	Exposure	Obser.	ps	as	an	pv	oc	or
Control		7752	0.013 ±0.013	0	0	0.077 ±0.032	0.013 ±0.013	0
20 to 53	16;22	1602	0.125 ±0.090	0	0	0.69 ±0.21	0.062 ±0.062	0.062 ±0.062
44 to 82	24;30	4652	0.086 ±0.043	0	0.022 ±0.022	0.37 ±0.09	0.086 ±0.043	0.022 ±0.022
68 to 105	24	1304	0.230 ±0.133	0.077 ±0.077	0.077 ±0.077	0.230 ±0.133	0.154 ±0.109	0
	30	1140	1.49 ±0.36	1.49 ±0.36	0.53 ±0.21	1.32 ±0.34	0.44 ±0.20	0
90 to 128	24	1866	0.81 ±0.21	1.18 ±0.25	1.23 ±0.26	13.89 ±0.80	1.61 ±0.29	0.32 ±0.13
	30	872	0.69 ±0.28	0.57 ±0.26	4.59 ±0.70	9.18 ±0.98	4.82 ±0.72	1.72 ±0.44
116 to 148	24	844	0.47 ±0.24	0.47 ±0.24	0.83 ±0.31	13.88 ±1.19	4.62 ±0.72	9.00 ±0.98

bristle, either in time or in amount of effect, or in both of these ways.

b. Discussion.

To determine whether the temperature effect was upon part or all of the population in this experiment, left-right correlation coefficients were calculated for each pair of bristles. The bristle frequencies were very low in the control series and in the first and second instar experimental series. No females in these series lacked both bristles of any pair. Beginning with the 30 hour exposure in the 68 to 105 hour period, such females appeared in the experimental series, and indicated marked left-right correlations for several of the bristles in this and succeeding periods. These coefficients were as follows: an, +0.634; pv, +0.564; oc, +0.270; and or, +0.452. The t value for each coefficient was very large, indicating clearly a deviation from 0. From the size of the coefficients it seems certain that the temperature effect was not the same on the entire population, and that only a part of the population was affected by the temperature so far as the bristles are concerned. It is possible that her, as in the case of the male ps and as bristles, we may be dealing with a special sensitive period like Child's TEP. In these females, however, the TEP differs from his in that it does not appear to have exactly the same position in late development for any two of the bristles.

Correlation coefficients were also calculated for each of the two-by-two combinations of the an, pv, oc, and or bristles in thsi experiment. There appeared to be no dif-

ference in the rough data between the 90 to 128 and 116 to 148 hour periods; and they were therefore grouped for these correlation calculations. The an bristle showed a positive correlation with the oc bristle but no correlation with the pv and or bristles; the pv bristle was correlated with the or bristle. All of these coefficients were of approximately the same size, ranging from +0.19 to +0.22, with t values between 8 and 9. They indicate that these bristles tend to be affected together in the sc/v car females. In this respect these data differ from those of their sib males which did not show such tendencies.

c. Comparison with sib males.

The data of the 36°C. sc/v car females in Table 38 may be compared with the sib male data in Table 28 (page 99). They are alike in showing probably the same general period of high sensitivity to the temperature treatment in respect to the ps and as frequencies, and a decrease in pv frequency in every developmental period. The frequency change, however, is oppositely directed in the ps and as bristles between the sexes. And in the effects upon the other bristles the data are opposed to each other. The bearing of these observations on the problem of sc dominance will be discussed later.

d. Comparison with 40°C. sc/v car.

These data may also be compared with those of the 40°C. sc/v car females in Table 37 (page 120). The two series differ in conditions in that the 36°C. series was raised

on Moldex food. That may be the reason for the slight difference in pv frequencies in the controls. In their comparable developmental periods the data resemble each other to the extent of showing, in each case, affected ps, as, and pv frequencies. But otherwise they differ markedly in bristles affected and in amount of effect. Since the preliminary experiment on non-Moldex food showed comparable results in the 35 to 36°C. temperature range it seems probable that the differences between the 40 and 36°C. data are due to the difference in temperature treatment.

4. The effects of 36°C. on wild-type flies.

a. Introduction.

In December of 1937, I tested the effects of nearly lethal exposures to 36°C. on the bristle frequencies of the wild-type stock synthesized from the sc and v car stocks, considering the same bristles as in previous experiments. This synthetic wild stock contained the normal allele of sc from the v car stock and the normal alleles of v and car from the sc stock. The rest of its genes were in unknown proportions from the two stocks. The Y-chromosome was from the v car stock; and the original egg cytoplasm was from the sc stock. This wild stock was inbred without selection for 13 generations, and then continued by mass matings without selection for an additional year and a half before this experiment.

The conditions and plan for this experiment were the same as those in the other 36°C. experiments. The wild

stock, however, could not withstand 30 hour exposures to 36°C. satisfactorily. Exposures were 12 to 24 hours in length.

b. The male data.

The data showing the effects of the 36°C. treatment on the synthetic wild-type males are given in Table 39. The temperature effected a decrease in ps and as frequencies throughout larval life. An especially pronounced decrease followed the 24 hour exposure during the 72 to 107 hour period. The an frequency was probably unaffected. (There was but one missing bristle in all the treated males) The pv and oc bristles were affected mainly during the 72 to 107 hour period. The or frequency was affected during that and the following period.

c. Discussion.

During the periods of pronounced temperature effect, there were positive left-right correlations between the affected bristles in wild-type males, indicating that the temperature did not affect all of the population similarly. In the case of the ps bristles there was no correlation in the control and first and second instar series. In the remaining periods the correlation was as follows: 72 to 94 hours, +0.366; 72 to 107 hours, +0.336; 96 to 124 hours, +0.189; and 120 to 154 hours, +0.142. The only correlation in the as bristles appeared in the 72 to 107 hour period, when the coefficient was +0.336. The pv bristles showed a correlation of +0.15 in the same period. The or bristles showed a correlation in the combined 72 to 107 and 96 to 124

TABLE 39.

Wild Type ♂♂ - 36°C. - Larval and Young Pupal Exposures -

Period	Exposure	Obser.	ps	as	an	pv	oc	or
Control		3936	1.20 ±0.18	0.46 ±0.11	0	0	0.10 ±0.05	0.03 ±0.03
24 to 55	12;24	1592	3.83 ±0.48	1.38 ±0.29	0	0	0	0
48 to 81	12;24	2442	2.94 ±0.34	2.42 ±0.31	0	0.08 ±0.06	0	0
72 to 94	12	768	13.80 ±1.25	6.14 ±0.87	0	0	0	0.13 ±0.13
72 to 107	24	1008	50.50 ±1.58	39.40 ±1.54	0.10 ±0.10	2.38 ±0.48	0.69 ±0.26	1.29 ±0.36
96 to 124	12	1328	4.06 ±0.54	4.82 ±0.59	0	0.38 ±0.17	0.23 ±0.13	0.68 ±0.23
120 to 154	12;24	934	1.39 ±0.38	1.07 ±0.34	0	0.11 ±0.11	0	0.11 ±0.11

hour periods of +0.18. The t values of these coefficients ranged from 3 to 8. The an and oc bristles showed no left-right correlation.

There proved to be positive correlations between some of the bristles in the 72 to 107 hour period. This amounted to +0.265 (t value of 6) between the ps and as bristles; to +0.112 (t value of 2.5) between the as and oc bristles; and to +0.104 (t value of 2.3) between as and or. These coefficients suggest a tendency for these bristles to be affected together in this experiment.

The period of marked effect of the temperature for all the bristles was in the 72 to 107 hour period. Presumably the 24 hour exposure affected more larvae than did the 12 hour exposure; it may also have affected some larvae through a longer portion of a hyper-sensitive period, such as appeared in the earlier 36°C. experiments. It may be that the hypersensitive period is shorter for wild-type males and more like that found by Child in sc at viable temperature.

d. Comparison with 36°C. sc males from sc x v car.

The data from the 36°C. wild-type males may be compared with the summarized data from the 36°C. sc males from sc x v car in Table 38 (page 122). It is to be kept in mind that all of the frequency changes in the wild-type are decreases and that the direction of change in sc males is indicated by the sign of the coefficient of change. It can be seen that the greatest differences in the two experiments are in the oppositely directed effects of the temperature on the ps, as, and or frequencies, the first two of which are

especially strongly affected in both experiments.

The comparison of these data will be discussed later with respect to their indications concerning the responses of sc and modifiers to the temperature treatment.

e. The female data.

The data from the 36°C. wild-type females are given in Table 40. They show that the bristle frequencies were affected to some extent in every developmental period, and notably following a 24 hour exposure during the 72 to 107 hour period. The main effect appeared in the ps and as frequencies, which showed a maximum decrease during the above period. The an bristle was unaffected at all periods. The pv frequency was affected only slightly, during the third instar and early larval periods. The oc was also only slightly affected, during the 72 to 107 hour period. The or frequency was affected during the pupal period only.

f. Discussion.

Left-right correlation coefficients indicate that the temperature effect was not on the entire population with respect to some of the bristles. The ps bristles showed a correlation of +0.30 (t value of 7) in the 72 to 107 hour period, and a correlation of +0.514 (t value of 12) in the 120 to 154 hour period. The high coefficient in the latter period, coupled with the low frequency, suggests that the temperature effect was upon the few larvae present rather than upon some of the many pupae. The as bristles showed a correlation of +0.22 (t value of 5) in the 72 to 107 hour

TABLE 40.

Wild Type ♀♀ - 36°C. - Larval and Young Pupal Exposures -

Period	Exposure	Obser.	ps	as	an	pv	oc	or
Control		3832	0	0	0	0.03 ±0.03	0	0.03 ±0.03
24 to 55	12;24	1498	0.07 ±0.07	0.13 ±0.09	0	0	0	0
48 to 81	12;24	2200	0.18 ±0.09	0.05 ±0.05	0	0	0	0
72 to 94	12	608	0.49 ±0.28	0	0	0	0	0
72 to 107	24	966	10.13 ±0.97	2.90 ±0.54	0	0.10 ±0.10	0.21 ±0.15	0
96 to 124	12	1934	0.36 ±0.14	0.16 ±0.09	0	0.10 ±0.07	0	0.26 ±0.12
120 to 154	12;24	1022	1.27 ±0.35	0.39 ±0.20	0	0.29 ±0.17	0	1.17 ±0.34

period. The or bristles showed a correlation of +0.38 (t value of 12) in the 96 to 124 hour period, and a correlation of +0.16 (t value of 4) in the 120 to 154 hour period. The high value of the 96 to 124 hour coefficient for the or bristles, coupled with the low frequency, suggests that the temperature effect was upon the few pupae present in these cultures, rather than upon a few of the many larvae.

g. Comparison with wild-type males.

A comparison of the female data of Table 40 with the wild-type male data of Table 39 (page 127) reveals a sexual dimorphism both in the control and in the experimental bristle frequencies. The males have fewer bristles (i.e., have more bristles missing) both at 25°C. and in the series exposed to 36°C. In the control series, the difference is evident only in the ps and as bristles; but in the treated series it extends to the other bristles in the period of greatest effect.

This dimorphism is typical of many sex-linked characters. Bar and alleles (see Review of Literature) show such a behavior; and Child's data show it clearly for sc. Payne and Plunkett had already reported that sc females have more bristles than do sc males. Although no bristle counts were made in the females in my experiments, it was obvious that sc females showed greater numbers of the bristles used here. It was frequently necessary to examine many flies to find one female with all the bristles missing. The dimorphism present in this synthetic wild stock suggests

that part of the cause of missing bristles is due to one or more sex-linked genes. No attempts have been made to locate the gene or genes concerned.

There is an additional difference between the male and female data which is of interest; namely, in the sensitive period of the or bristles. It has been argued that this period lies in the pupal stage in wild-type females. In the males it appears to lie in the third instar. Although no attempts were made to determine accurately the relative lengths of the egg-larval periods in the two sexes in this race of flies, it is reasonably certain that there was no such great difference as there is between the maxima of decrease in or frequency.

h. Comparison with 36°C. sc/v car females.

A comparison may be made between the wild-type female data of Table 40 and the 36°C. sc/v car female data of Table 38 (page. 122). It is evident that while the two sets of data resemble each other in respect to the general period of highest sensitivity late in the third instar, they differ widely in relative amount of effect. The ps and as bristles are relatively more affected in the wild-type females; the pv, oc, and or bristles are relatively more affected in sc/v car females. There appears to be no significant difference between the two types of females at 25°C.

The bearing of these observations on the problem of the dominant effect of sc will be discussed in the next part of this report.

5. Effects of heterozygous sc at 36°C.

It has been noted that at 25°C. sc shows practically no dominant effect in respect to the six bristles considered in these experiments. The bristle frequencies are practically identical in sc/v car and wild-type females. There are differences in the bristle frequencies between these two types of females at 36°C., however. By comparing these differences with the differences between the wild-type males and the sc males from sc x v car, it is possible to suggest the direction of the dominant effect of sc at 36°C. To make this comarison possible, it is necessary to assume that flies from the sc x v car cross and flies of the wild-type race derived from that cross carry essentially the same bristle modifiers, so that the only effective difference between the two types is with respect to the sc gene itself.

The data of the sc males in Table 28 (page 99), of their sib sc/v car females in Table 38 (page 122), of the wild-type males in Table 39 (page 127), and of their sib wild-type females in Table 40 (page 130) are presented in a much simplified summary in Table 41. Each entry in this table represents the direction of change in bristle frequency in these 36°C. flies when compared to their respective 25 to 26°C. controls, the change being evident in one or more developmental periods. A plus sign indicates an increase, a minus sign a decrease, in bristle frequency. In the case of the oc bristle in sc males, the entry of both signs indicates that there was an increase in one period and a decrease in another. When the two types of flies of the same sex showed a similarly directed frequency change, the lesser of

TABLE 41.

Reactions of sc and Modifiers to 36°C.

Table	Type of Fly	ps	as	an	pv	oc	or
39	wild ♂	-	-	0	(-)	-	-
28	sc ♂	+	+	0	-	±	+
40	wild ♀	-	-	0	(-)	(-)	(-)
38	sc/v car ♀	(-)	(-)	-	-	-	-

the two has been enclosed by parentheses in Table 41.

In wild-type males, the ps and as frequencies decreased greatly at 36°C. In sc males they increased greatly. in wild-type females and in sc/v car females they decreased; but the decrease was less in sc/v car than in wild type. This suggests that the 36°C. change in sc effect, evident so strongly in males, is probably also responsible for the lesser decrease in sc/v car females. In other word, sc acts as a slight dominant in heterozygous females in the same direction in which it acts as a recessive in sc males, so far as these bristles are concerned.

The an frequency did not change in either type of males nor in wild-type females. It decreased, however, in sc/v car females. If a decrease, proportionately as small, had occurred in sc males, it could easily have escaped attention, because of the already very low an frequency. This decrease in sc/v car females can therefore be interpreted as a dominant effect of sc at 36°C. which is not necessarily different in direction from its homozygous effect in sc males at 36°C.

The pv frequency decreased in all four types of flies; but it decreased considerably more in sc males and sc/v car females than in wild type flies. The dominant effect of sc in this case seems to be in the same direction as its homozygous effect.

The oc frequency decreased very slightly in the third instar in both sexes of 36°C. wild-type flies. In the sc/v car females it decreased slightly in the first, second, and first half of the third instars; and considerably more in

the last half of the third instar and in the early pupal period. In sc males, the oc frequency increased in the second instar; and did not change in the first half of the third instar. In the second half of the third instar it decreased following a 24 hour exposure and increased following a 30 hour exposure, both changes being small but statistically significant. It decreased in the early pupal period. With respect to this bristle frequency, the effects of heterozygous sc, following the 36°C. treatment, seem to be different from the effects of homozygous sc in males. The difference is more pronounced in the second instar; it may disappear entirely in late development.

The or frequency decreased in both sexes of wild-type flies, somewhat earlier in males than in females, following the 36°C. treatment. In sc males it increased in every developmental period. In the heterozygote, it decreased late in development, considerably more strongly than in the wild-type females. In this case, too, the dominant effect of sc seems to differ from its male homozygotic effect, but this time late in development, rather than early.

From the above considerations it seems probable that the ultimate effect of sc is so altered by nearly lethal exposures to 36°C. that it affects the bristle frequencies when in heterozygous condition; but that it does not necessarily affect them all in the same direction as when it is in homzygous condition in sc males. The differences in direction of effect, apparent in the or bristles and suggested in the oc bristles, may be due to the difference in sexes.

Child, however, did not find a difference in direction of effect of viable temperatures on any of the several bristles when comparing males with females.

Another fact of interest suggested by these comparisons is that the temperature effect is upon the sc-dependent reaction, or reaction system, and not jsut upon modifier reactions which the presence of sc makes visible.

The data from sc and wild-type males suggest also that in the cases of the ps, as, and or bristles, the effects of sc following the high temperature treatment are opposite to the effects of the modifiers; and that the sc effect is much the dominant one in each case.

6. Summary and conclusions.

(1) During embryonic, late larval, or early pupal development, nearly lethal exposures to 40°C. effect decreases in ps, as, and pv bristle frequencies in sc/v car females. The oc frequency is also reduced during the embryonic and late larval periods.

(2) During any one of several selected periods in larval and early pupal development, nearly lethal exposures to 36°C. effect a decrease in one or more of the bristle frequencies in sc/v car females.

(3) The ps, pv, and oc frequencies decrease in all tested periods.

(4) The as and an frequencies decrease in the late third instar and early pupal periods.

(5) The or frequency decreases in the late third instar and in early pupal periods.

(6) All the bristles show periods of maximum effect late in development; but they differ among themselves as to the periods or exposures which produce the maxima.

(7) During any one of several selected periods in larval and early pupal development nearly lethal exposures to 36°C. effect a decrease in one or more bristle frequencies in males and females of a wild-type stock derived from the sc and v car stocks used in earlier experiments.

(8) The ps and as frequencies decrease throughout larval life in both sexes.

(9) The an frequency is unaffected in both sexes.

(10) The pv frequency decreases in the third instar in both sexes.

(11) The oc frequency decreases only slightly in both sexes, the decrease appearing in the third instar series.

(12) The or frequency decreases in the third instar in males, and in the early pupal period in females.

(13) In all bristles the decreases appear to be greater in males than in females.

(14) All frequencies (with the exception of that of the female bristle) show a decrease in the third instar.

(15) The response of sc males to the 36°C. treatment is opposite to that of wild-type males with respect to the ps, as, and or frequencies.

(16) The effects of the 36°C. treatment are more pronounced in sc/v car females than in wild-type females with respect to the an, pv, oc, and or frequencies; and are less pronounced with respect to the ps and as frequencies.

(17) The data are discussed with reapect to a dominant effect of sc in sc/v car females.

(18) At 25°C. sc is not dominant in any of the bristles considered.

(19) Following the 36°C. treatment sc is probably dominant to some degree in all of the bristles considered; but its effect as a dominant may not always be in the same direction as its homozygotic effect in sc males.

(20) The effect of the 36°C. treatment is upon the effect of sc as well as upon the effects of modifiers.

(21) In respect to the ps, as, and or bristles in males, the effect of sc following the 36°C. treatment is dominant to the effects of the modifiers.

H. Interpretations of the Experimental Results.

In presenting the data in previous sections of this report, little discussion has been made of their probable theoretical significance. There are a number of questions which may be asked along this line, and we may turn now to the answers to some of them.

1. The meaning of the change in bristle frequency.

One of the first questions to be considered is, "What does a bristle frequency mean?"

Child (1936) has attempted to answer that question, and his reasoning applies here as well as ~~in~~ to his experiments. He pictures bristle formation as an instance of the classical "all-or-none" principle. The bristle is either formed or not formed, in any given instance; part bristles are not found. This suggests that a certain threshold concentration of bristle-forming substance must be present to insure the formation of a given bristle. An amount just beneath this threshold value will produce no bristle; an amount any distance above it will result in the formation of but one bristle. In any individual fly the presence or absence of a given bristle can be thought of as being determined by the presence or absence, at the time of bristle formation, of an amount of bristle-forming substance equal to or exceeding the threshold concentration.

The bristle frequency, however, is determined by observations on a population of individuals. It is Child's view that in a population, the amount of bristle-forming substance available for a given bristle is not the same in

every fly. Instead it is distributed at random around a mean concentration, in greater or lesser amounts to the many individuals, the distribution following the probability-integral curve. The mean bristle frequency of a population represents the point on the distribution curve which coincides approximately with the threshold concentration of substance necessary for bristle formation. The frequency indicates the proportion of the population in which the amount of bristle-forming substance available for the bristle equals or exceeds the threshold concentration.

From this reasoning it can be seen that a race of flies which manifests 100% presence of a given bristle is one in which the mean amount of substance available for bristle formation is so far above the threshold level that normal variations from individual to individual do not produce any individuals with a sub-threshold concentration. Similarly, the complete absence of a given bristle in a population indicates that the mean amount of substance available for bristle formation is far below the threshold. A race of flies which shows a mean bristle frequency between 0 and 100% for a given bristle is one whose mean concentration of bristle-forming substance is in that case nearer the threshold concentration.

On this view it is logical to interpret the effects of both environmental and genetic factors on mean bristle frequencies as representing changes in the mean concentrations of bristle-forming substances. The gene sc in homozygous condition shifts the mean concentration considerably below the threshold level in the case of the bristles considered in the experiments discussed in this report. It seems probable that

the effect of larval density, Moldex, genetic modifiers, and temperature is to shift these same mean concentrations nearer or farther away from the threshold, depending upon whether the indicated change is an increase or a decrease in frequency of bristles present. In the experiment with wild-type flies, the mean concentration was so far above the threshold level that the only practically measurable change was a decrease in mean concentration which resulted in more individuals with missing bristles.

2. Nature of the temperature effect on a bristle.

A second question which may be asked is, "What is the nature of the temperature effect on a bristle?"

A large amount of literature has been published on the effects of temperature on living matter (see Bělehrádek 1935). The general indications are that temperature affects the chemical and physical processes which constitute the life functions of the individual. This it does, not by creating new chemical reactions, as a rule, but by increasing the rates of reactions already taking place to a greater or lesser extent, and by destroying substances such as enzymes, proteins, etc., so as to make impossible the reactions in which they have an integral part.

If the temperature effect on sc bristles reported here were simply a change in the rate of a reaction which is of some importance to the ultimate amount of bristle-forming substance to be produced, then it would be expected that an increase in the length of the exposure would produce a measurable increase in the observed effect. With the possible exception of the results from 36°C. exposures on the frequencies

of ps and as bristles, this is not the case in these experimental results. Rather, we are confronted with a temperature effect, which, once it has manifested itself, does not appear to change with increasing length of exposure. It seems probable, therefore, that we are not dealing with a temperature effect on a simple chemical reaction.

If the temperature effect on sc bristle frequencies results from a destruction of an enzyme or some other necessary substance, then it might be expected that the effect would appear after a given exposure, more or less as and instantaneous shock. Providing the effect is complete in all members of the population, it would not appear to increase with an increase in length of exposure. This is, in fact, what is observed in these experiments. It seems logical, therefore, to offer destruction of an enzyme or some other necessary substance as a more probable explanation of these high temperature effects. This interpretation of the results carries more weight by reason of the fact that 36°C. and 40°C. are in the temperature range in which such destructive processes are known in some cases to take place.

3. Meaning of differences in bristle responses to temperature.

A third question which may be asked is, "What is the meaning of the differences between the response of the several bristles to temperature treatments?"

Child (1935) suggested that differential temperature effects on the smae chemical reaction or substance in different parts of the fly - due to differences in length of TEP or of rate of reaction, or to both of these - could produce

all the differences observed in his experiments. The unity of the developmental positions of the short TEP's for all of the bristles made such an interpretation plausible.

In my experiments with 36 and 40°C., however, the many variations in position as well as in duration of the TEP's make such an interpretation, in general, too simplified. Only the ps and as bristles parallel each other sufficiently in response to the temperature treatment to allow such an interpretation. While they may have a more identical history than the other bristles, there is no conclusive evidence that such is the case. Certainly, on the whole, these temperature data suggest an independence of developmental history. And they do also suggest that critical reactions take place at many periods in development for each of the several bristles, possibly differing in nature from bristle to bristle.

4. Developmental history of sc.

A fourth question which may be asked is, "What do these results at 36 and 40°C. indicate concerning the developmental history of sc?"

It has already been suggested that the results of these experiments indicate the probability of several reactions being involved at different times, possibly different for the several bristles. Powsner (1935) has developed the theory of Plunkett (1932) that development consists of a long series of interacting chains of reactions, leading from the embryonic period through the larval period and finally to more specific and less inter-related reactions which form the imaginal characters. These reactions are dependent upon

environmental conditions as well as genetic constitution. In a very real sense, every adult organ is dependent upon the successful completion of some reaction at any given time in development; and it is dependent in such a way that the failure of this reaction, either because of environmental or genetic influences, or both, results in an alteration of the subsequent adult organ. This is an extremely general picture; and it provides the basis for a description of practically any type of observed phenomena, as Powsner realized. But the temperature effects on sc bristles, demonstrated in my experiments, are so diversified that for the present, at least, only such a broad and general interpretation can, I believe, be made of them.

It appears, then, from the temperature effects, that the normal development of sc-affected bristles is dependent upon the successful completion of one or more reactions taking place at any given time in development, from early embryonic life until the visible formation of the bristles from the trichogenic cells observed by Robertson (1936) in early pupal development.

5. Meaning of the viable temperature TEP's.

A fifth question which may be asked is, "What do the results of these experiments suggest concerning the general concept of TEP as developed from viable temperature studies on Drosophila mutant characters?"

In view of the considerable contrast between the results of Child's experiments and those of the experiments reported here, in respect to the position and duration of the TEP of the sc genotype, it seems probable that the results of

viable temperature studies on Drosophila mutants in general (see Review of Literature) can not be interpreted to indicate the period of most important developmental history for the character in question; and certainly not necessarily the only period when the character in question can be influenced by a change in developmental temperatures. The viable temperature TEP's can be said to indicate in each case the presence of a reaction or reactions which are of importance to the organ in question and which have temperature characteristics differing from those of development as a whole in the viable temperature range. In the cases of Bar, vestigial, and bent wing, where the sib wild types have been worked with in each case, the viable TEP's may also be considered to indicate a temperature influence on the ultimate effect of the gene on the adult character. That the TEP's happen to coincide in some cases with visible embryological processes does not necessarily mean a direct connection between them, as was pointed out by Miss Auerbach (1936).

The results of these high temperature experiments with sc suggest that experiments similar to these with characters such as Bar or vestigial might show as much broader TEP's for these characters as has been shown for sc.

6. Activity of the scute gene.

A sixth question which may be asked is, "What do these results suggest concerning the activity of the sc gene?"

These data are not critical on the direct action of the gene. Nor are any of the TEP data critical on this point. It can not be determined from TEP data if a given gene acts before the time of the TEP, so that the effects of temperature

are on certain of its products; or if the gene acts following the TEP and hence upon temperature affected materials; or if the temperature effect is upon the direct action of the gene itself during the indicated TEP.

In the case of sc, the comparative data of the 36°C. experiments with sc and wild-type males which have probably similar modifying genes do suggest strongly, however, that the high temperature treatment influences the ultimate effect of sc itself and not just the effects of modifiers which the presence of sc makes visible.

Digest of the Thesis

(1) The literature concerned with temperature-effective period studies on a number of Drosophila mutant characters is reviewed. It indicates, in general, a specific period in development in which changes in temperature can alter the effects of a given mutant gene.

(2) Data from scute males given nearly lethal treatments with supra-viable temperatures of 40 or 36°C. show that the phenotypic expression of this gene may be changed to some extent at practically any time in development, from the laying of the egg to the appearance of the bristle cells early in pupal development. This is in contrast to the brief period of sensitivity found for this phenotype by Child at viable temperatures. The nature of the effect depends upon (a) the temperature treatment given, (b) the bristles considered, (c) the bristle modifiers present in the scute stock, and (d) the developmental period in which the treatment is given.

(3) Data from scute males, from heterozygous scute females, and from both sexes of a closely related wild-type stock, all given the 36°C. treatment, show that scute has a dominant effect on some of the bristles under these conditions; and that the temperature alters both the effects of the scute gene and of the non-scute modifying genes on the bristles considered.

(4) These data support the theory of Plunkett, et al, that the phenotypic expression of a Mendelian character is dependent upon the successful completion of a series of reactions extending throughout development.

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