THE EFFECTS OF HIGH TEMPERATURE ON BRISTLE FREQUENCIES IN SCUTE AND WILD-TYPE MALES OF DROSOPHILA MELANOGASTER

PHILIP TRUMAN IVES[1]
California Institute of Technology, Pasadena, California

Received December 1, 1938

INTRODUCTION

DURING the past twenty-five years there has appeared a considerable amount of literature concerned with temperature-effective period studies on mutant races of *Drosophila melanogaster*. Adult characters which have drawn the most attention have been the compound eye, the wing, and the bristles. Most of the work on the eye has been done with Bar and its alleles (see especially DRIVER 1926 and 1931, LUCE 1931, and MARGOLIS 1935). Wing studies have been confined largely to vestigial and its alleles (see especially RIEDEL 1934, STANLEY 1935, HARNLY 1936, HARNLY and HARNLY 1936, and LI and TSUI 1936). The bristle studies have been on the bristle-removing genes, Dichaete (PLUNKETT 1926) and scute (CHILD 1935 and 1936, IVES 1935). It has been found to be general in most of these studies that under the conditions of the experiments the adult expression of these several mutants is affected by a change in developmental temperature over the viable temperature range (14° to 31°C) during only a limited developmental period. This period has come to be known as the temperature-effective period (TEP) of the mutant character.

This paper reports a series of experiments on scute (*sc*) using brief exposures to lethal temperatures (36° and 40°C). A preliminary account of part of this work has already been published (IVES 1935).

EXPERIMENTAL METHODS

The methods used in these experiments were described in the preliminary account. It has since been found, however, that the variation in experimental conditions (food and temperature) was enough to change bristle frequencies in the controls so that more than rough comparisons of data collected several months apart are unreliable. This accounted for many of the irregularities of the earlier report. Accordingly, all comparisons have since been made between sib control and experimental flies which were raised under as similar conditions as possible. Special attention was given to the number of larvae growing together in a culture, to the age of the parents, and to temperature and food conditions.

[1] The Rufus B. Kellogg University Fellow of Amherst College, Amherst, Massachusetts.

Flies in part of the experiments were raised on moldex-treated food, (BRIDGES 1937). The moldex affected some of the bristle frequencies, possibly by reducing the supply of yeast. It did not appear to affect the temperature responses of the bristles.

PLAN OF THE EXPERIMENTS

Experiments were carried out with *sc* males of two types: from *sc* females against *sc* males, and from *sc* females against vermilion (*v*) carnation (*car*) males; and with males of a wild type stock derived from the above *sc*-by-*v car* cross. The *sc* and *v car* stocks were inbred previous to beginning the experiments. Left-right correlations (PLUNKETT 1926) of the bristles considered were throughout the experiments negligibly small (less than 0.1) in both types of *sc* males in the control lines.

Experiments were carried out with both of the above *sc* matings at 40°C and at 36°C; and with the wild-type flies at 36°C, exclusive in each case of the first hour (the "a" exposure) during which the food temperature was rising from the control temperature of 25°C. The longest exposures were generally just under the limit of viable exposure for the majority of the culture population in each test. Variation in food temperature from culture to culture was mostly between 40 and 41°C, and 36 and 37°, respectively. The control food temperature, though constant to within about 0.2° for each section of an experiment, varied between 25 and 26° from section to section, with most sections between 25 and 25.5°. Controls were raised for each section individually.

The bristles considered in these experiments were as follows: anterior and posterior scutellars (AS and PS), anterior notopleurals (AN), postverticals (PV), ocellars (OC), and anterior and medial orbitals (OR). As reported by CHILD (1935), it was found that the last of these bristles could not be distinguished as to anterior or medial position in *sc* males and that they were practically never present together on the same side of the fly. (They were observed so together but once in some 500,000 half-fly observations connected with these experiments.) They have therefore been grouped as OR in these experiments as they were in those of DR. CHILD.

Bristle frequencies have been computed on the basis of the half-fly unit (PLUNKETT 1926). A difference between two frequencies has been considered significant when it equals or exceeds twice its standard error. In the data to be discussed, most of the differences between control and experimental frequencies are as large as four times their standard errors. To obtain low standard errors generally not less than 1000 half-fly observations were made for each length of exposure tested. The controls were generally considerably larger in number.

The experiments were designed to test the effects of graded lengths of

exposures to high temperatures during a number of developmental periods covering most of the development preceding the visible formation of bristles (ROBERTSON 1936). Thus, in the 40° experiments, exposures ranged from half an hour to three hours in length and tested a part of each of the egg, three larval, and pupal stages of development. In the 36° experiments two lengths of exposures were used, and each of the larval instars and the early pupal period were tested. The developmental period in each case has been calculated in hours from the beginning of the egg-laying period. Its spread represents the sum of the egg-laying and exposure periods. It is known that there was variation in developmental time for the larvae of a culture population, making the calculated time only approximate. It probably represents the position of the considerable majority of the larvae, since late-hatching flies were not classified for bristles.

No attempt was made to determine exactly the relation of the tested developmental periods to such physiological landmarks in development as the hatching of the egg, the larval molts, and pupation. But it was noted that pupation (cessation of larval movement and eversion of anterior spiracles) took place in the control series at an approximate mean of 120 hours after egg laying. From a number of studies on the time interrelations of the above stages of development (see POWSNER 1935 for one) the following general time chart is suggested for practical use here:

Egg stage	0 to 24 hours
First larval instar	24 to 48 hours
Second larval instar	48 to 72 hours
Third larval instar	72 to 120 hours
Pupal stage	120 hours on.

A comparison of this chart with the tables of data to be presented will indicate the developmental stage during which the indicated treatment was given.

THE EXPERIMENTAL DATA

The 40°C experiments

The data for the *sc* males in the 40° experiment with the *sc*-by-*v car* mating are summarized in table 1 as frequencies of bristles present per 100 half-flies. Where no effect of the temperature treatment was apparent following any of the exposures, all of the exposure series, from "a" through three additional hours of 40°C, have been added together. When a temperature effect was apparent, the data from the shortest exposure to show the effect and the data from all longer exposures have been added together. Thus each frequency represents a mean of several groups of data. The shortest exposure included in each frequency is indicated in each case

above the frequency. As a general rule, increasing the length of the exposure did not increase a temperature effect, once the latter had been established. Left-right correlations were similar to their respective controls, less than 0.1, and not statistically different from 0, indicating that the effect was upon the population as a whole. With each experimental frequency is given the corresponding control frequency and the standard

TABLE 1

sc ♀♀ × *v car* ♂♂—*40°C.—Bristles present per 100 half-males.*

GROUP	PS	AS	AN	PV	OC	OR
0 to 10 hours	a	a	a	a	a	a
(a—2 hours)	0.08	2.39	0.82	0.58	11.28	12.84
Control	0.29	2.60	0.22	0.43	3.39	7.13
S.E. of diff.	0.17	0.64	0.29	0.29	1.03	1.18
20 to 30 hours	a	a	a	1½ hrs	½ hr.	½ hr.
(a—3 hours)	0.36	2.49	0.71	0.22	8.53	7.50
Control	0.52	2.39	0.57	0.62	4.53	4.87
S.E. of diff.	0.09	0.20	0.11	0.11	0.39	0.38
48 to 58 hours	a	a	a	½ hr.	½ hr.	a
(a—3 hours)	0.24	1.44	0.39	0.09	9.23	8.83
Control	0.39	2.23	0.43	0.30	3.52	5.55
S.E. of diff.	0.11	0.27	0.13	0.09	0.42	0.47
72 to 82 hours	a	a	a	a	a	1 hr.
(a—3 hours)	0.26	2.24	0.78	0.08	9.95	4.95
Control	0.11	2.12	0.11	0.22	5.37	6.88
S.E. of diff.	0.10	0.38	0.13	0.11	0.63	0.67
96 to 106 hours	a	a	a	a	½ hr.	a
(a—3 hours)	0.25	2.03	0.53	0.30	5.70	4.68
Control	0.36	2.26	0.40	0.31	3.50	5.81
S.E. of diff.	0.12	0.29	0.13	0.11	0.40	0.45
120 to 130 hours	a	a	a	a	a	1 hr.
(a—3 hours)	0.13	1.97	0.34	0.61	6.81	5.20
Control	0.29	2.60	0.22	0.43	3.39	7.13
S.E. of diff.	0.15	0.47	0.15	0.21	0.61	0.78

error of the difference between the two frequencies (S. E. of diff.). Under the heading "Group" are included the developmental period during which treatment was given, and in parentheses under that, the minimal and maximal exposures used. In the 0 to 10 hour and 120 to 130 hour groups only even hour exposures were used; but in the others the length of exposure time was increased by half-hour steps.

The data of table 1 show clear effects of the temperature treatment on the OC and OR frequencies in each of the several developmental periods

tested. The OC frequency was increased in each period. The OR frequency was increased in the egg and first two instar periods and was lessened in the two-third instar periods and in the early pupal period. The frequencies of the other bristles are so small in the control data that small changes in them in either direction are not easily detectable, especially when the frequencies are interpreted in relation to a threshold amount of bristle-forming substance (CHILD 1936). It is apparent, however, that large changes (such as will be noted in the 36° experiment in the cases of the PS and AS frequencies) were not brought about by the 40° treatment. The apparent small changes in frequencies of PS and AS bristles are probably not significant, for when the six exposure groups comprising each frequency are considered individually their differences from their controls in each instance are not consistent in direction. The experimental frequency varies above and below the control, apparently at random. This is not true in the case of the PV frequency which, beginning with the one and one half hour exposure in the 20 to 30 hour group and with the half hour exposure in the 48 to 58 hour group, was consistently below the control frequency in each of the succeeding higher exposures. It appears likely, therefore, that this represents a real effect of the temperature. The AN frequency was consistently different from the control in only the 0 to 10 and 72 to 82 hour groups. In each case the control frequency was lower than usual, making the temperature effect a doubtful one.

Correlation coefficients were calculated for the one-by-one combinations of frequencies represented in table 1. None of them were significantly different from zero. While the correlation coefficient does not give an accurate measure of the intensity of association of bristles with each other, the absence of correlation here probably indicates the same sort of independence of one bristle from another as was found by CHILD.

Following the *sc*-by-*v car* experiment, a similar but less extensive series of tests was run with the *sc*-by-*sc* mating. There appeared to be consistent differences in some of the control frequencies in the two types of *sc* males. The changes from the *sc*-by-*v car* frequencies in bristles present per 100 half-males were in the order of from 0.6 to 0.06 for the PS bristles, from 3.3 to 0.6 for the AS, and from 5.2 to 12.1 for the OR, when several representative control series of different population densities were averaged together for each mating. There did not appear to be significant differences in the other frequencies. The differences noted here maintained themselves later in a test of the two matings raised together in time and under similar environmental conditions.

The data from the *sc*-by-*sc* 40° experiment are summarized in table 2 in a form similar to that of table 1. Exposures of less than one hour beyond a were not used. The exposures included in each section of the data are

indicated under the statement of the developmental period tested. One hour exposures were not tested in the 48 to 58 and 72 to 82 hour groups. In the 96 to 106 and 120 to 130 hour groups each of the tested exposures was lethal to many larvae and pupae, making necessary the summation of the three exposures in each case to secure significant numbers of observations.

TABLE 2

sc ♀♀ × *sc* ♂♂—*40°C.—Bristles present per 100 half-males.*

GROUP	PS	AS	AN	PV	OC	OR
0 to 10 hours (1 hour)	0.14	0.97	2.50	1.53	4.73	13.48
Control	0.07	1.11	0.81	0.88	2.50	9.93
S.E. of diff.	0.16	0.46	0.63	0.52	0.90	1.51
20 to 30 hours (1; 2; 3 hours)	0	0.73	1.45	1.00	8.04	20.92
Control	0	0.37	0.57	0.63	3.86	16.97
S.E. of diff.		0.25	0.33	0.30	0.78	1.28
48 to 58 hours (2; 3 hours)	0	0.42	1.00	0.25	6.28	14.98
Control	0	0.11	0.82	0.71	4.24	13.52
S.E. of diff.		0.20	0.33	0.20	0.78	1.16
72 to 82 hours (2; 3 hours)	0.30	1.59	4.78	1.33	5.74	11.89
Control	0.13	1.05	0.98	1.16	2.78	7.47
S.E. of Diff.	0.11	0.27	0.43	0.25	0.49	0.69
96 to 106 hours (1; 2; 3 hours)	0.27	2.22	3.91	1.16	9.68	6.04
Control	0.27	1.64	1.54	0.51	5.63	5.87
S.E. of diff.	0.18	0.50	0.63	0.35	0.98	0.83
120 to 130 hours (1; 2; 3 hours)	0	0.83	1.28	0.60	4.53	5.14
Control	0.04	0.56	0.91	0.37	5.03	5.06
S.E. of diff.	0.02	0.27	0.33	0.22	0.63	0.66

As in the *sc*-by-*v car* experiment, the clearest effects of the temperature in the data of table 2 are in the OC and OR frequencies. The OC frequency was increased in all but the early pupal period. The OR frequency was increased through the early third instar period; but it was unaffected in the later third instar and early pupal periods. The AN frequency was also affected in this mating, being increased in the egg, first instar and third instar periods. The PV frequency was affected, if at all, during only the second instar, when it appears to have been lessened. As in the *v car*

mating, the PS and AS frequencies did not appear to be significantly affected at any time.

A comparison of the data of tables 1 and 2 indicates small but probably significant differences in response to temperature treatment with respect to the AN, PV, OC, and OR frequencies—mainly in duration of the 40° TEP. The PV, OC, and OR TEP's are longer in the *v car* mating; the AN TEP is longer in the *sc*-by-*sc* mating. Differences will be shown to exist also in the responses of the two types of *sc* males to 36°C.

The 36°C experiments

Preliminary tests at 36° with the *sc*-by-*v car* mating showed that exposures as brief as four hours during the first and second instars produced no significant changes in bristle frequencies. Subsequent experiments were made with much longer exposures. The results of these tests are summarized in table 3. The most significant frequency changes are those of the PS and AS bristles. These frequencies were increased during each of the larval periods tested, although the affect on the PS frequency is on the border line of significance in the second instar. The AN frequency was not affected at any time. The PV frequency was lessened during every period tested. The OC frequency was very irregularly affected. It was unchanged in the first instar and in the first half of the third instar. In the second instar it was very strongly increased. During the late third instar period it appears to have been lessened by the 24 hour exposure and increased by the 30 hour exposure in each case by three times the Standard Error of the difference. In the early pupal period it was again considerably lessened. The OR frequency was increased during every period, the increase in the late third instar series appearing only in the 30 hour group.

It is evident in the data of table 3 that the effects on the PS and AS frequencies were most pronounced during the third instar series; and that the 30 hour exposures produced a greater change than did the 24 hour exposures. The first of these observations suggests a period of greater sensitivity, perhaps related to CHILD'S viable temperature TEP. Left-right correlation coefficients of these bristles in these series were comparatively high, being between 0.2 and 0.5 as compared to control coefficients of less than 0.1. These coefficients are high enough to indicate that the temperature effect was not uniform in all the larvae so far as the PS and AS bristles were concerned. They suggest, too, that the higher frequencies of the longer exposure groups are the result of more larvae having been affected by the temperature with respect to these bristles.

When the data are compared with those of table 1, the 40° data from the same mating, it is clear that there is a fundamental difference in the affects of nearly lethal exposures to the two temperatures on some if not all of

the bristles considered. The differences are greatest in the responses of the PS, AS, and OC bristles. The differences between the controls in these two series are due partly to the inclusion of moldex in the culture media and

TABLE 3

sc ♀ ♀ ×*v car* ♂ ♂—*36°C.—Bristles present per 100 half-males.*

GROUP	PS	AS	AN	PV	OC	OR
24 to 53 hours (16; 22 hours)	2.43	14.00	1.93	1.07	9.86	6.78
Control A	0.76	4.26	1.60	1.98	10.58	3.27
S.E. of diff.	0.47	1.08	0.51	0.48	1.17	0.83
44 to 74 hours (24 hours)	1.67	8.22	1.11	0.43	12.90	4.02
Control B	1.13	5.38	1.72	1.51	6.52	1.78
S.E. of diff.	0.41	0.86	0.40	0.32	1.01	0.57
45 to 82 hours (30 hours)	1.49	10.54	1.88	0.63	22.50	6.17
S.E. of diff. from control A	0.41	1.03	0.53	0.45	1.45	0.83
68 to 101 hours (24 hours)	7.26	12.88	2.58	0.53	10.22	6.36
Control C	0.90	5.64	2.42	1.64	10.61	2.69
S.E. of diff.	0.73	1.02	0.52	0.30	0.99	0.73
68 to 105 hours (30 hours)	16.39	22.25	2.26	0.68	11.48	4.83
S.E. of diff. from control A	1.03	1.27	0.54	0.45	1.22	0.76
92 to 126 hours (24 hours)	12.35	15.50	3.46	0.47	7.49	2.81
Control D	0.99	6.57	2.97	1.41	10.63	2.29
S.E. of diff.	0.83	1.05	0.58	0.32	0.95	0.52
90 to 128 hours (30 hours)	20.44	22.75	2.18	0.27	14.84	8.31
S.E. of diff. from control C	1.51	1.60	0.60	0.29	1.41	1.06
116 to 148 hours (25 hours)	2.04	4.69	1.80	0	6.25	6.62
S.E. of diff. from control C	0.52	0.81	0.53	0.22	1.00	0.91

partly to a control temperature of 26° during the most of the 36° experiment. Preliminary experiments at 35 to 37° with non-moldex food and at a control temperature of 25° indicated the same sort of temperature re-

sponses as in table 3. The differences between the 40° and 36° bristle responses are due therefore to differences in the nature of the effects of the two temperatures and not to differences in other experimental conditions.

TABLE 4

sc ♀♀ × *sc* ♂♂—*36°C.—Bristles present per 100 half-males.*

GROUP	PS	AS	AN	PV	OC	OR
15 to 34 hours (6; 12 hours)	0.04	0.67	1.55	0.84	6.87	16.66
Control	0.09	0.41	0.63	0.68	5.47	13.25
S.E. of diff.	0.07	0.22	0.30	0.26	0.71	1.05
24 to 57 hours (12; 24 hours)	0.08	1.23	1.70	0.39	5.01	16.96
Control	0.06	0.17	0.61	0.61	3.09	8.34
S.E. of diff.	0.10	0.32	0.40	0.25	0.73	1.23
48 to 80 hours (12; 24 hours)	0	0.35	1.48	0.21	4.43	7.94
Control	0	0.07	0.70	0.42	2.23	8.93
S.E. of diff.		0.18	0.39	0.21	0.67	1.04
72 to 92 hours (12 hours)	1.23	5.82	0.49	0.49	8.28	6.56
Control	0	0.59	1.61	0.46	8.38	15.83
S.E. of diff.	0.32	0.70	0.46	0.29	1.23	1.43
72 to 107 hours (24 hours)	7.82	16.84	0.60	0.34	4.90	5.50
Control	0	0.59	2.10	0.45	4.06	8.42
S.E. of diff.	0.79	1.05	0.60	0.31	0.99	1.27
96 to 116 hours (12 hours)	0.22	3.99	3.99	0.11	3.66	4.88
Control	0.15	0.26	0.70	0.50	2.92	8.08
S.E. of diff.	0.17	0.66	0.67	0.16	0.69	0.86
120 to 140 hours (12 hours)	0.15	0.30	0.89	0.30	3.26	6.07
Control	0.15	0.26	0.70	0.50	2.92	8.08
S.E. of diff.	0.16	0.23	0.39	0.24	0.74	1.03

Following the above 36° experiment a similar experiment was carried out with the *sc*-by-*sc* mating, the data from which are summarized in table 4. This mating could not stand 30 hours at 36° at any period of development. Exposures as brief as three hours proved fatal in early embryonic development; and 24 hours was intolerable during the 96 to 116 and 120 to 140 hour periods. The data show that the only significant effects on the PS frequency occurred during the first half of the third instar.

The AS frequency was increased during the second and both third instar periods. The frequencies of these bristles in the controls, however, was so low that lesser increases such as indicated in the *v car* cross in table 3 would probably not have been detected. As in that mating, the temperature effect here was greatest for these bristles during the third instar and during its longer exposure. The AN frequency was increased during the first and second instars, and during the late third instar. It was lessened during the first half of the third instar. The PV frequency was lessened during the late third instar, and apparently unaffected at other times. The OC frequency was increased during the first and second instars, and unaffected in later periods. The OR frequency was increased during the first instar; it was not significantly affected during the second instar; and it was lessened in the third instar and early pupal periods.

A comparison of these data with those of table 3 indicates differences in the responses of the two types of *sc* males to 36° which are clear in the cases of the AN, PV, OC, and OR bristles, and which may also extend to the PS and AS bristles. Considered together with the similar comparisons of the data of tables 1 and 2 and of their respective controls, which were made above, they suggest strongly the presence of genetic modifiers of *sc* which differ in the *sc* and *v car* stocks and which determine to some extent the type of temperature response observed.

A comparison of the data of tables 4 and 2 indicates, as did the similar comparison between tables 1 and 3, a difference in the responses of genetically similar *sc* males to 36° and 40°. The differences in this case, too, extend to some extent to every bristle considered.

It is worthy of note here that the differences in the effects of 36° and 40° on either type of *sc* male are greater than are the differences in response of the two types of *sc* males to either temperature. For example, the data of tables 4 and 2 resemble each other much less than do the data of tables 4 and 3, with respect to the temperature effects indicated. Thus, in these experiments the temperature treatment given appears to be more important than the genetic modifiers present in determining the resultant bristle-frequency response. Whether this would be true in experiments involving *sc* males with other genetic modifiers can not be stated. Even here the statement is not equally applicable to all the bristles considered. The AN bristle response in table 4 resembles much more that in table 2 than it does that in table 3, considering the several developmental periods as a group. This indicates that the modifiers are of more importance for the AN bristle in these experiments. For the other bristles, however, the temperature treatment appears to be the stronger determining factor in the nature of the response.

After these experiments with *sc* males a 36° experiment was carried out

with a wild-type stock which contained the normal allele of *sc* from the *v car* stock and the normal alleles of *v* and *car* from the *sc* stock. The autosomes were in non-regulated proportions from the two stocks. The cytoplasm was from the *sc* stock. The data from the males of this experiment are summarized in table 5. Each bristle frequency per half-male was so

TABLE 5

Wild-type ♂♂—36°C.—Bristles present per 100 half-flies.

GROUP	PS	AS	AN	PV	OC	OR
Control	98.90	99.54	100	100	99.90	99.97
24 to 55 hours (12; 34 hours)	96.17	98.62	100	100	100	100
S.E. of diff.	0.51	0.31				
48 to 81 hours (12; 24 hours)	97.06	97.58	100	99.92	100	100
S.E. of diff.	0.38	0.33		0.06		
72 to 94 hours (12 hours)	86.20	93.86	100	100	100	99.87
S.E. of diff.	1.26	0.88				0.13
72 to 107 hours (24 hours)	49.44	61.60	100	97.62	99.31	98.71
S.E. of diff.	1.59	1.54		0.48	0.26	0.36
96 to 124 hours (12 hours)	95.94	95.18	100	99.62	99.77	99.32
S.E. of diff.	0.57	0.60		0.17	0.14	0.23
120 to 154 hours (12 hours)	98.61	98.93	100	99.89	100	99.89
S.E. of diff.	0.42	0.36		0.11		0.11

high that an increase in frequency in the treated lines would not have been apparent. A decrease in frequency was detectable, however. It can be seen from the table that there was a significant decrease in the PS and AS frequencies in each of the larval period series, with a relatively very large decrease in the third instar series. For practical considerations this effect is the reciprocal of the effect on *sc* males of comparable genetic background shown in table 3. With respect to the other bristles, the only apparent effect of the treatment occurred in the third instar series, in the period of greatest effect on the PS and AS frequencies. With the exception of the AN frequency, which was unaffected in either experiment, the other bristle frequencies do not appear to bear any respective relation to each other in their temperature responses in tables 3 and 5.

DISCUSSION

The data of tables 1 through 5 indicate the kinds of changes produced in bristle frequencies in Drosophila males by nearly maximum exposures to temperatures above the viable range. The data of the first four of these tables indicate that such changes may be produced in *sc* males for one bristle or another during practically any period of development previous to visible formation of the bristles in the young pupa. The data of table 5 indicate that some of the bristle frequencies of wild-type flies are also affected by such treatment, some of them in a direction opposite to the effect in *sc* males.

Comparisons of the data of tables 1 through 4 with each other have been discussed above. They make clear the following observational conclusions. The nature of the temperature response in bristle frequencies in *sc* males depends upon (1) the temperature treatment given, (2) the developmental period during which the treatment is given, (3) the modifiers present in the treated stock, and (4) the bristles which are considered.

Since we do not know the chemistry of bristle formation these data can not be discussed with reference to specific substances involved. They must therefore be discussed in terms of a general and theoretical developmental scheme. A number of such schemes have been suggested.

The non-existence of a pattern relationship of the bristles in *sc* such as developed by DUBININ (1929) and others, and by STURTEVANT and SCHULTZ (1931) was pointed out by CHILD (1935). His results have been confirmed by calculations from the data in both control and treated lines in some of these experiments. Similarly confirmed is CHILD'S evidence of the non-existence of such a physiological gradient scheme as proposed by GOLDSCHMIDT (1931). The bristles of the *sc* pattern appear to develop independently of each other in *sc* flies.

A very general theory, currently known as the "rate theory," was proposed by PLUNKETT (1926) and has since been developed and applied by him (1932) and others in interpreting the effects of both genes and environment upon the adult characters of an organism. (See POWSNER 1935 for diagrammatic illustration.) Based upon orthodox concepts of physical chemistry and the general concepts of genetics, this theory postulates that both genes and environmental forces (such as temperature) affect a given phenotype (such as that of *sc*) by changing the rate or duration, or both, of processes—physical, chemical, biological—involved in the development of the phenotype. The effects on duration, which can be, to some extent, directly measured, are interpreted as being determined in turn, by effects on rates of other developmental processes.

The data of the above tables can be explained on such a broad and generalized theory; but the number of arbitrary assumptions which must

be made for a detailed analysis is too great to make such an analysis profitable at this time. Using the response of the OC bristle in the 40° experiments and the responses of the PS and AS bristles in the 36° experiments of both *sc* and wild-type males, the author, for his own satisfaction, has worked out most of the details for one such schematic analysis in each case, with the aid of DR. CHILD. The above data, while not useful at present as confirmatory evidence for the rate theory, are not in conflict with it.

A comparison may be made between the TEP's found in these studies and the TEP found in CHILD'S experiments. The difference, as already noted, is very marked, since in this case the TEP varies distinctly from bristle to bristle and occupies every part of bristle development for one or another of the bristles. In CHILD'S experiments the TEP was approximately the same for all bristles at all temperatures studied, being limited to the latter half of the third instar when a culture population was considered. The simplest explanation of this apparently fundamental difference between the two groups of data is that many more of the reactions involved in bristle formation are susceptible of effective change by high temperature treatments than by viable temperature treatments. In fact, the differences in the data from the two types of treatment—and especially the considerably more complex nature of the data from high temperature treatments—may be interpreted as indicating the presence of more developmental reactions concerned with bristle formation than one is led to suspect from the data of the viable temperature studies alone. CHILD'S rather strictly limited TEP led him to conclude that the effect of temperature was in the main on the same reaction at each bristle site, and that small differences between bristle locations with respect to rate or duration of the temperature-affected reaction satisfactorily account for the observed differences in amount and direction of change in the bristle frequencies. It can not be shown that the same reasoning does not apply to the results of these high temperature studies. But the data picture is so much more complex—marked differences in TEP position and length, both between bristles and between the 36° and 40°C experiments—that such an explanation seems too simple in this case. The results suggest rather that we are probably concerned here with other reactions which are not detectably affected by changes within the viable temperature range. We may carry this analysis a step further and suggest that the differences in bristle frequency responses to 36°C as compared to the respective responses to 40°C indicate that the reactions detected by the two treatments are for most of the bristles different within themselves. Thus we may consider each of the three treatments—viable temperature, 36°C, and 40°C—as being specific for certain reactions which respond detectably to it and not detectably, if at all, to either of the other two.

GOLDSCHMIDT (1938) has intimated that such a comparison of viable and lethal temperature data is not permissible because the high temperature treatment is probably "destructive." This is a generally accepted interpretation of high temperature effects on living matter and many substances associated with it. However, biochemical studies on such substances as enzymes generally reveal "destructive" or "inactivating" temperatures to be considerably above those used in this series of experiments. And in the particular case in point this criticism does not carry so much weight as it may at first seem to. The variations in bristle frequencies which are brought about by the two types of treatment, with viable temperatures and with lethal temperatures, are not markedly different in magnitude, considering them as a whole. And while the effect of high temperature, especially of 40°C, seems to cause a reaction to go to completion within one or two hours, or even less (no increase in effect with increase in length of exposure), the temperature-effective period for the individual fly through the viable temperature range is itself not a long time. It may be that in both cases we are dealing with reactions which take place quickly and that there is no difference in the strictly temperature effect. On the whole, the differences between the data from the two types of treatment are not such as to suggest at present that in one case the effect of temperature is preponderantly "destructive" and in the other "constructive," in whatever sense one chooses to use those terms with reference to the bristles considered. It seems better, for the present at least, to interpret the differences between the viable and lethal temperature data as has been done in the preceding paragraphs.

Studies upon mutant character TEP's appear to have been made originally with the hope of detecting periods of specific gene activity. It is clear, however, that such a direct relation between the two can not be determined by methods thus far reported. The effects of the temperature treatment may be upon reactions whose components or products are later used in reactions controlled by a given gene or its products; or they may be directly upon such gene controlled reactions; or they may be upon subsequent reactions involving the products of such gene controlled reactions. The TEP is only the time in development during which such an effect is produced; it does not indicate which of the above types of effect is being produced. It is the time when the phenotype is changed by the experimental technique used, the relation of the change to specific gene action remaining obscure. This is as true for CHILD'S TEP as it is for the TEP's detected by higher temperatures. It is also true for all TEP studies thus far reported.

It may be argued that the temperature effects on *sc*-affected bristles are not on the effects of the *sc* gene (in any of the three ways outlined

above); but that instead they are upon the effects of other bristle-frequency-modifying genes which the presence of the extreme modifier, *sc*, makes visible. When the bristle frequency is interpreted on the basis of CHILD'S (1936) application of the threshold principle this argument is strengthened. In the absence of *sc* such modifiers might well be unable to move the amount of bristle-forming substance detectably nearer to the threshold amount necessary for bristle formation, when a population of flies is considered. A comparison of the data of table 3 with those of table 5 sheds some light on this point. In the one case the data are from *sc* flies, in the other from non-*sc* flies. From the comparison of *sc* males of the *sc*-by-*v car* mating with those of the *sc*-by-*sc* mating evidence was obtained for the presence of genetic modifiers of *sc*. PAYNE (1920) found two such modifiers. Doubtless there are others. In addition, we know that there are many mutant genes which affect bristle frequencies. Some of these are known to respond to viable temperature changes in their bristle effects. It is probable that their normal alleles do also exert some influence on bristle frequencies. We may consider, then, that for practical purposes the differences between the temperature responses of *sc* males in table 3 and the closely related wild-type males in table 5 are due to the effects of temperature on *sc* plus the group of modifiers in table 3 and on the modifiers alone in table 5. The wild-type allele of *sc* is here considered only as one of a large group of modifiers and not necessarily any more important in its temperature response than any of the other wild-type alleles of known bristle-affecting mutant genes. From the differences between the two tables it is clear not only that there is a marked effect of temperature on the ultimate effect both of the *sc* gene itself and of the modifiers as a group but also that the response of *sc* is opposite in direction to the response of the modifiers as a group, at least for the bristles most obviously affected. The strength of this statement is lessened perhaps by the fact that we can not determine, from these data at least, how much of this difference is due to possible qualitative effect of *sc* on the temperature response of the modifiers, individually or as a group.

ACKNOWLEDGMENTS

This report is adapted from part of the author's Thesis for the Doctorate Degree (1938) at the California Institute of Technology. It is a pleasure to acknowledge here the aid of the members of the Department of Biology at that Institute, particularly that of PROFESSOR ALFRED H. STURTEVANT under whose direction this work was done, and of the members of the Department of Biology at Amherst College, particularly that of DR. GEORGE P. CHILD.

SUMMARY

(1) Bristle frequencies were tabulated for two races of *sc* males treated with nearly maximum exposures to 40°C and to 36°C, and for closely related wild-type males treated with 36°C, the treatments varying in length and being given at various times in egg-larval-pupal development.

(2) In the 40° experiments the OC frequency was increased during nearly all of bristle development; the AN, PV, and OR frequencies were less extensively affected; and the PS and AS frequencies were unaffected.

(3) In the 36° *sc* experiment the scutellar frequencies were increased during the larval period and especially in the third instar. The other bristles were less extensively affected.

(4) In both 36° and 40° *sc* experiments there were consistent differences in both control bristle frequencies and in frequency responses to the temperature treatment between males from a *sc*-by-*v car* cross and those from a *sc*-by-*sc* cross.

(5) In the wild-type 36° experiment the scutellar frequencies were lessened in each tested larval period and especially in the third instar. The other bristle frequencies were only slightly affected.

(6) The effects of 40° and probably of 36° on bristle frequencies do not appear to be increased by lengthening the exposure period beyond that needed to establish the effect in the entire population.

(7) The nature of the effects of temperature on the bristle frequencies of *sc* males depends upon the temperature used, the developmental period treated, the genetic modifiers present, and the bristles considered.

(8) The absence of correlation between bristles in both control and treated lines confirms the results of CHILD in demonstrating the nonexistence of "pattern" relationship or "gradient systems" in *sc*.

(9) The data are explainable in terms of the "rate theory" of gene and environmental effects on developmental processes, but are not critical for the theory itself.

(10) Extensive differences between the results following the 40°, 36°, and CHILD'S viable temperature treatments are interpreted as indicating that probably different reactions are affected detectably by each of the three treatments.

(11) The data do not indicate that the effects of high temperature are necessarily different in nature from those of viable temperature.

(12) The TEP is the time when the phenotype is changed, but it does not indicate the relation of the change to specific (mutant) gene action.

(13) The differences between the 36° effects on *sc* and wild-type males are interpreted as indicating that the temperature affects the ultimate bristle effect of both *sc* and the group of modifying genes, and that in this

case the direction of the effect on *sc* is opposite to that on the other bristle modifiers as a group with respect to the bristles most clearly affected.

LITERATURE CITED

BRIDGES, C. B., 1937 Revised data on culture media and mutant loci of *Drosophila melanogaster*. Tab. Biol. **14**: 343–353.

CHILD, G. P., 1935 Phenogenetic studies on *sc-1* of *Drosophila melanogaster*. I. The association between bristles and the effects of genetic modifiers and temperature. Genetics **20**: 109–126.

1935 II. The temperature-effective period. Genetics **20**: 127–155.

1936. III. The effect of temperature on *sc-5*. Genetics **21**: 806–816.

DRIVER, E. C., 1926 The temperature-effective period—the key to eye-facet number in Drosophila. J. Exp. Zool. **46**: 317–332.

1931 Temperature and gene expression in Drosophila. J. Exp. Zool. **59**: 1–28.

DUBININ, N. P., 1929 Allelomorphentreppen bei *Drosophila melanogaster*. Biol. Zbl. **49**: 328–339.

GOLDSCHMIDT, R., 1931 Die entwicklungsphysiologische Erklärung des Falls der sogennanten Treppenallelomorphe des Gens scute von Drosophila. Biol. Zbl. **55**: 535–554.

1938 Physiological Genetics. ix+375 pp. McGraw-Hill Book Co. New York and London.

HARNLY, M. H., 1936 The temperature-effective periods and the growth curves for length and area of the vestigial wing in *Drosophila melanogaster*. Genetics **21**: 84–103.

HARNLY, M. H., and HARNLY, MARIE L., 1936 The effects of the gene on growth and differentiation as shown by the temperature responses of pennant and its heterozygote in *D. melanogaster*. J. Exp. Zool. **74**: 41–59.

IVES, P. T., 1935 The temperature-effective period of the scute-1 phenotype. Proc. Nat. Acad. Sci. **21**: 646–650.

LI, J. C., and TSUI, Y. L., 1936 The development of vestigial wings under high temperature in *Drosophila malanogaster*. Genetics **21**: 248–263.

LUCE, W. M., 1931 The temperature-effective period in infra-Bar and its heterozygotes. J. Exp. Zool. **59**: 467–498.

MARGOLIS, O. S., 1935 Studies on the Bar series of Drosophila. I. The effect of the gene vestigial on facet number in Bar. Genetics **20**: 156–171.

1935 II. The temperature-effective period for facet determination in Bar and Bar-vestigial. Genetics **20**: 207–222.

PAYNE, F., 1920 Selection for high and low bristle number in the mutant strain, "reduced." Genetics **5**: 501–542.

PLUNKETT, C. R., 1926 Interaction of genetic and environmental factors in development. J. Exp. Zool. **46**: 181–244.

1932 Temperature as a tool in phenogenetics: methods and results. (Abstract). Proc. Sixth Int. Cong. Genetics **2**: 158–160.

POWSNER, L., 1935 The effects of temperature on the duration of the developmental stages of *D. melanogaster*. Physiol. Zool. **8**: 474–520.

RIEDEL, HILDA, 1934 Der Einfluss der Entwicklungstemperatur auf Flügel-und Tibialänge von *Drosophila melanogaster* (wild, vestigial und die reziproken Kreuzungen). Arch. f. Entw-Mech. **132**: 463–503.

ROBERTSON, C. W., 1936 The metamorphosis of *Drosophila melanogaster* including an accurately timed account of the principal morphological changes. J. Morph. **59**: 351–399.

STANLEY, W. F., 1935 The effect of temperature upon wing size in Drosophila. J. Exp. Zool. **69**: 459–495.

STURTEVANT, A. H., and SCHULTZ, J., 1931 The inadequacy of the sub-gene hypothesis of the nature of the scute allelomorphs of Drosophila. Proc. Nat. Acad. Sci. **17**: 265–270.