FLY SIZE, EMERGENCE TIME AND STERNOPLEURAL CHAETA NUMBER IN *DROSOPHILA*

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1. INTRODUCTION

In the literature, there are observations in *Drosophila melanogaster* on the effect of environment on chaeta number. For example, Plunkett (1926) studied various genes affecting chaeta number, and showed that under conditions of increased competition, chaeta number was reduced. Similarly, as the temperature at which flies were grown was increased from 14° C. to 30° C., a general decrease in chaeta number occurred. He found no effect in his wild-type stock, but, as his initial experiments were done on a small number of chaetæ on each side of the fly, this is not surprising. More recently, data on sterno-pleural chaeta number have been collected at 20° C., 25° C., 30° C. and a fluctuating temperature running smoothly from 30° C. at midday to 20° C. at midnight, and back to 30° C. at midday (Thoday, 1958, quoting Beardmore) for the F_1 and subsequent generations from Oregon and Samarkand inbred lines. At 20° C., chaeta number was higher than at 25° C., which in turn was higher than at 30° C. The fluctuating temperature (20/30° C.) gave about the same number of chaetæ as at 25° C., as might be expected. After the flies were placed in their new environment, further changes occurred in subsequent generations. At 20° C., chaeta number increased further, and at 30° C. decreased further. This progressive change from generation to generation was taken to indicate that chaeta number is a character of adaptive significance. Similarly, sternopleural chaeta asymmetry decreased over the generations in the 30° C. and 20/30° C. environments, this suggesting that sterno-pleural symmetry is of adaptive significance.

Further evidence that sterno-pleural chaeta number is of adaptive significance is presented by Mather (1953), Tebb and Thoday (1954), Thoday (1955) and Beardmore (1960), and in other publications.

It seems, therefore, reasonable to report some results for flies grown at two levels of competition on various concentrations of the tyrosinase inhibitor phenyl-thio-carbamide (P.T.C.) at 25° C. P.T.C. was shown by Parsons and Kroman (1960) to reduce the size of flies, and appeared to have no other morphological effect. An additional smaller experiment will be reported where flies grown at 30° C. are compared with those grown at 25° C. Thus it is possible to study relations between fly weight, which is a measure of fly size, and sterno-pleural chaeta number by growing flies on increasing concentrations of P.T.C. and at

different temperatures. That there is probably some relation between fly weight and chaeta number is indicated by Reeve and Robertson's (1954) results on sternite chaeta number where they found that the male fly had fewer chaetæ than the female, and the reduction seemed to be related to the surface area of the fly, which is of course related to fly weight. This sex difference applies to sternopleural chaetæ also (Mather, 1953). Reeve and Robertson (1954) also found a correlation between fly size, as measured by thorax length, and chaeta number for flies grown under variable culture conditions. Mann (1923) found that flies carrying the gene sternopleural that developed slowly had fewer chaetæ than those developing more rapidly, and that the reduction in chaeta number was related to fly size. Preliminary results showing a correlation between chaeta number, surface area, and fly weight for various genotypes under different environmental conditions have been recently reported (Gibson, Parsons and Spickett, 1961).

2. METHOD

Various numbers of replicates in 4″ vials were set up for the three genotypes, Oregon–R (++), ebony″ (*e″e″*), and Oregon × ebony heterozygotes (*e″*+) at 2 levels of competition, namely 25 and 100 newly-hatched larvæ per vial on a series of concentrations of P.T.C. at 25° C. For brevity, we shall refer to these 2 levels of competition as low and high levels. At high concentrations of P.T.C., the number of replicates was increased, in an attempt to obtain adequate data for meaningful results, since fewer flies were expected to emerge. The adults were scored daily as they emerged for fly weight and sternopleural chaeta number. A torsion balance accurate to 0·02 mgm. was used to measure fly weight.

A second smaller experiment was set up at 25° C. and 30° C. using the Oregon stock on a few levels of P.T.C., to obtain information on the effect of temperature on fly weight and chaeta number. In this experiment the high level of competition had 150 larvæ per replicate, and the low level 25 larvæ per replicate.

3. FLY WEIGHT AND P.T.C. CONCENTRATION

In fig. 1 graphs of mean fly weight are given for each sex and each level of competition, for the 3 genotypes ++, *e″*+, and *e″e″* on various concentrations of P.T.C. The points on these graphs have necessarily different accuracies, depending on the number of flies classified. However, they are adequate to show any trends. A few points at the highest concentrations are omitted since very few flies emerged, and would confuse any trends. All the points plotted are means from 5 or more flies.

These graphs show clearly that (1) females weighed more than males at a given level of competition on a given concentration of P.T.C., (2) flies grown at the high level of competition weighed less than those grown at the low level for a given sex on a given concentration of P.T.C., and (3) fly weight decreased as P.T.C. concentration was increased. The greatest decrease in fly weight occurred on low concentrations of P.T.C. For *e″e″*, most of the decrease occurred between the control and 0·01 per cent. P.T.C. At 0·05 per cent. P.T.C., no *e″e″* flies survived as found by Parsons and Kroman (1960).

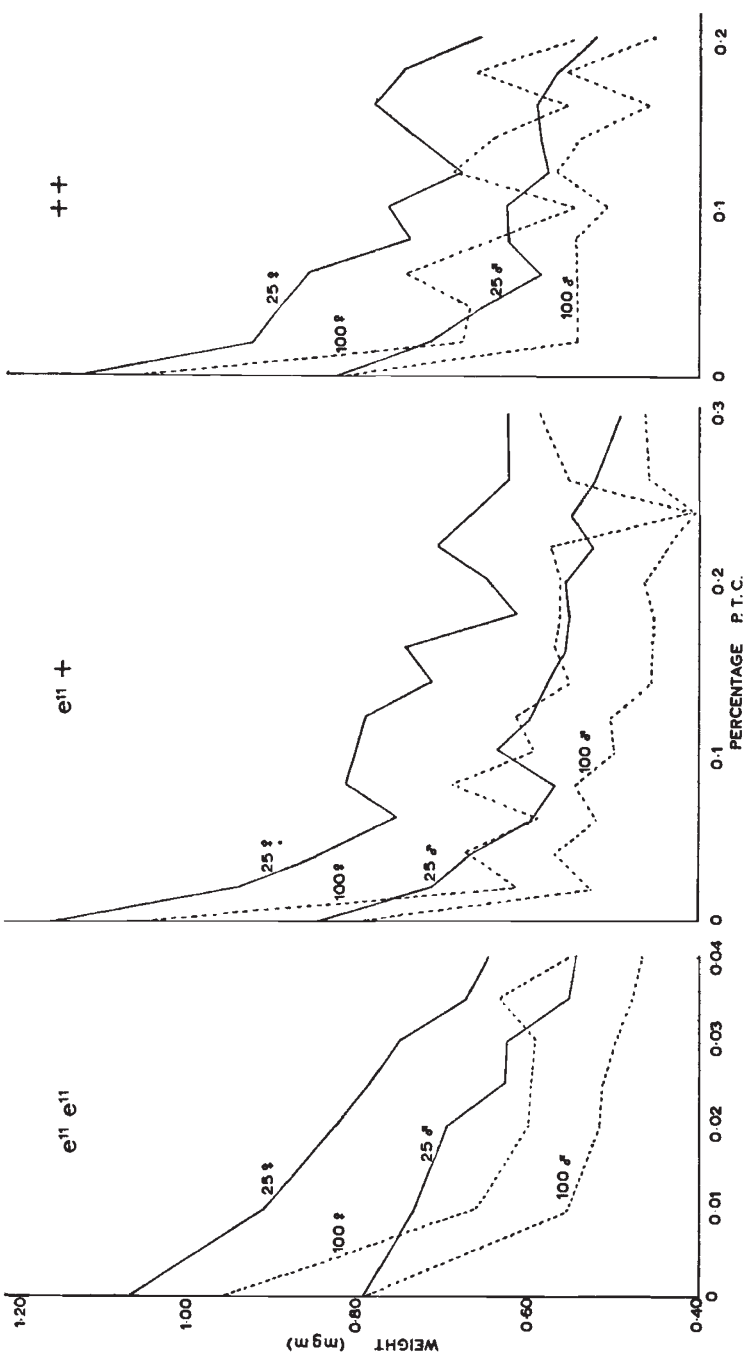

FIG. 1.—Mean fly weight at different concentrations of P.T.C. for the genotypes $e^{11}e^{11}$, $e^{11}+$, and $++$.

For $e''+$ and $++$, most of the decrease occurred between the control and 0·04 per cent. P.T.C. In this case, the lethal concentration was usually somewhat greater than 0·30 per cent. P.T.C. There was, however, a general tendency for fly weight to be slowly reduced at the higher concentrations of P.T.C. for all genotypes.

Coefficients of variation of fly weight were computed for some of the lower concentrations of P.T.C. (table 1). To avoid confusion, coefficients of variation are not given for P.T.C. concentrations where some, or all, of the entries in the table would be based on less than 30 flies.

TABLE 1

Fly weight and chaeta number variability

Larvæ per replicate	Fly weight coefficient of variation				Chaeta no. coefficient of variation				Chaeta no. asymmetry $\left(\frac{A}{T}\times 1000\right)$			
	25		100		25		100		25		100	
$e''e''$	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control . .	0·061	0·058	0·061	0·069	0·067	0·100	0·092	0·103	56	58	53	76
0·01 per cent. P.T.C.	0·102	0·077	0·081	0·120	0·084	0·082	0·104	0·107	57	51	65	35
0·02 per cent. P.T.C.	0·096	0·099	0·167	0·136	0·110	0·090	0·101	0·083	68	56	55	61
$e''+$												
Control . .	0·085	0·078	0·078	0·071	0·084	0·088	0·095	0·101	54	60	56	58
0·02 per cent. P.T.C.	0·113	0·083	0·110	0·114	0·081	0·094	0·073	0·097	56	54	45	49
0·04 per cent. P.T.C.	0·147	0·110	0·158	0·134	0·094	0·092	0·091	0·114	63	53	57	59
0·08 per cent. P.T.C.	0·123	0·170	0·173	0·149	0·081	0·088	0·097	0·087	59	46	42	59
0·10 per cent. P.T.C.	0·164	0·134	0·176	0·206	0·070	0·091	0·054	0·105	47	49	64	49
0·14 per cent. P.T.C.	0·234	0·204	0·200	0·220	0·077	0·083	0·086	0·087	52	49	51	55
0·16 per cent. P.T.C.	0·195	0·216	0·178	0·206	0·094	0·089	0·103	0·111	47	56	50	55
$++$												
Control . .	0·075	0·089	0·081	0·080	0·102	0·076	0·088	0·088	61	50	62	59
0·02 per cent. P.T.C.	0·127	0·091	0·144	0·152	0·097	0·079	0·091	0·092	46	39	57	50
0·04 per cent. P.T.C.	0·116	0·103	0·202	0·169	0·097	0·088	0·087	0·079	60	65	52	53

In all cases, coefficients of variation increased with P.T.C. concentration. Flies differed in size between sexes (fig. 1), but for a given treatment the coefficients of variation differed very little, so suggesting that the coefficient of variation assesses variability tolerably well, and is independent of the mean.

There was little difference in variability due to the levels of competition. Assuming that each coefficient of variation is equally accurate, which for $n>30$ is a fair approximation, an analysis of variance of the coefficients of variation can be done for each genotype to test possible effects of sex, competition and P.T.C. concentration. Multiplying the coefficients of variation by 1000 for convenience, we get the analyses of variance of the coefficients of variation as presented in table 2. For all genotypes, variation in P.T.C. concentration

is significant, and for the homozygotes, there is a suggestion of a competition effect, such that at the high level of competition, the coefficients of variation are slightly higher than at the low level.

TABLE 2

Analyses of variance of the coefficients of variation of fly weight presented in table 1

	d.f.	M.S.	P.
e″e″			
Levels of P.T.C.	2	3878·58	<0·05
Competition	1	1656·75	<0·20
Sex	1	6·75	>0·20
Competition × Sex	1	140·08	>0·20
Error	6	476·36	...
e″+			
Levels of P.T.C.	6	9543·29	<0·001
Competition	1	488·89	>0·20
Sex	1	54·32	>0·20
Competition × Sex	1	308·89	>0·20
Error	18	387·29	...
++			
Levels of P.T.C.	2	4655·08	<0·05
Competition	1	4294·08	<0·05
Sex	1	310·08	>0·20
Competition × Sex	1	6·75	>0·20
Error	6	682·64	...

If, as before, we regard the coefficients of variation as equally accurate, *i.e.* contributing equal information in the statistical sense, then we can add the 4 coefficients for each level of P.T.C., and do a regression analysis of these summed coefficients on P.T.C. concentration for each genotype. The regression coefficients obtained, with

TABLE 3

Significance of regression of coefficients of variation of fly weight on P.T.C. concentration

Genotype	Regression coefficient	Probability
e″e″	1·25	<0·05
e″+	0·31	<0·001
++	1·32	<0·20

levels of significance, are given in table 3, and indicate that as P.T.C. concentration was increased, the coefficients of variation increase in parallel.

All cultures were classified daily until emergence had finished to obtain total emergence figures. To compare these, percentage emergence is given in table 4 for the 3 genotypes. This is the easiest method

of comparison, since variable numbers of replicates were used during the course of the experiment. As might be expected, percentage emergence fell as P.T.C. concentration was increased. There was little difference in levels of competition. Parsons and Kroman (1960) found that more flies emerged at the high level than at the low level on 0·04 per cent. P.T.C. This effect does not occur consistently in the results reported here, so the phenomenon, although statistically significant in Parsons' and Kroman's (1960) data, apparently depends

TABLE 4

Percentage emergence

Larvæ per replicate	*e″e″*	
	25	100
Control	57	50·5
0·01 per cent. P.T.C.	72·5	47·5
0·02 per cent. P.T.C.	61·5	46·5
0·03 per cent. P.T.C.	9·33	28·67
0·04 per cent. P.T.C.	6	3·75

Larvæ per replicate	*e″*+		++	
	25	100	25	100
Control	90	76·5	91·5	83
0.02 per cent. P.T.C.	82·67	75	97·6	82·5
0.04 per cent. P.T.C.	69	75	90·67	52
0.08 per cent. P.T.C.	44·44	51·5	17·33	15·67
0·10 per cent. P.T.C.	47·25	38	29·16	25
0·14 per cent. P.T.C.	33·33	31·33	21·68	15
0·16 per cent. P.T.C.	31·5	29	12·75	19·25
0·20 per cent. P.T.C.	11·25	8·75	10·5	4·5
0·24 per cent. P.T.C.	12·25	5	4·5	0·5
0·30 per cent. P.T.C.	3·75	3	1·5	2·75
0·34 per cent. P.T.C.	1	1·25	0	0

considerably on the precise culture conditions. At lower concentrations (0·02 and 0·04 per cent. P.T.C.), there was no great difference in emergence between ++ and *e″*+, but at concentrations above 0·08 per cent. P.T.C., *e″*+ became heterotic as found by Parsons and Kroman (1960).

In table 5, means and coefficients of variation of emergence time (the time taken for newly hatched larvæ to emerge as adults) are given. Both increased as P.T.C. concentration increased, and in all cases they were larger at the high level of competition than at the low level. Parsons and Kroman (1960) reported similar results. Hence, correlated with decreasing fly weight and increasing variability of fly weight is a prolongation of emergence time.

4. CHAETA NUMBER AND P.T.C. CONCENTRATION

In fig. 2, chaeta number is plotted against P.T.C. concentration for each sex, level of competition and genotype. The graphs show (1) females had more chaetæ than males for a given level of competition and P.T.C. concentration, (2) flies at the high level of competition had fewer chaetæ than those grown at the low level for a given sex on a given concentration of P.T.C., and (3) chaeta number decreased as P.T.C. concentration was increased. The greatest decrease of chaeta number occurred on low concentrations of P.T.C.

TABLE 5

Means and coefficients of variation of emergence time

Larvæ per replicate	Emergence time (days)		Coefficient of variation of emergence time	
	25	100	25	100
e″				
CONTROL	7·63	8·01	0·066	0·093
0·01 per cent. P.T.C.	7·75	8·05	0·067	0·109
0·02 per cent. P.T.C.	8·64	10·23	0·061	0·109
e″+				
CONTROL	7·02	7·42	0·018	0·067
0·02 per cent. P.T.C.	7·06	7·69	0·039	0·077
0·04 per cent. P.T.C.	7·21	8·17	0·079	0·110
0·08 per cent. P.T.C.	8·91	9·79	0·132	0·124
0·10 per cent. P.T.C.	8·47	10·29	0·108	0·147
0·14 per cent. P.T.C.	9·45	11·35	0·083	0·198
0·16 per cent. P.T.C.	9·56	11·67	0·084	0·199
++				
CONTROL	7·11	7·52	0·044	0·086
0·02 per cent. P.T.C.	7·24	9·02	0·075	0·107
0·04 per cent. P.T.C.	7·98	10·57	0·097	0·136

Fly weight and chaeta number therefore react similarly to the various stresses. This suggests that the two variables are correlated. This possibility is discussed in section 5.

In table 1, coefficients of variation of chaeta number are given, but unlike fly weight no trend is obvious. Furthermore, the analyses of variance of the coefficients of variation showed no trends.

We can also study asymmetry of chaeta number between the 2 sides of the fly. Asymmetry has been measured as the summed absolute differences A between the 2 sides of a set of flies, divided by their total chaeta number T by Thoday (1955, 1958) and Beardmore (1960). This measure of asymmetry has been recently criticised by Reeve (1960), who maintains that there are always 3 macro-chaetæ on each side of the fly, hence we should divide by the total chaeta number minus 6. However, it is not possible in all flies to find 3 macro-chaetæ;

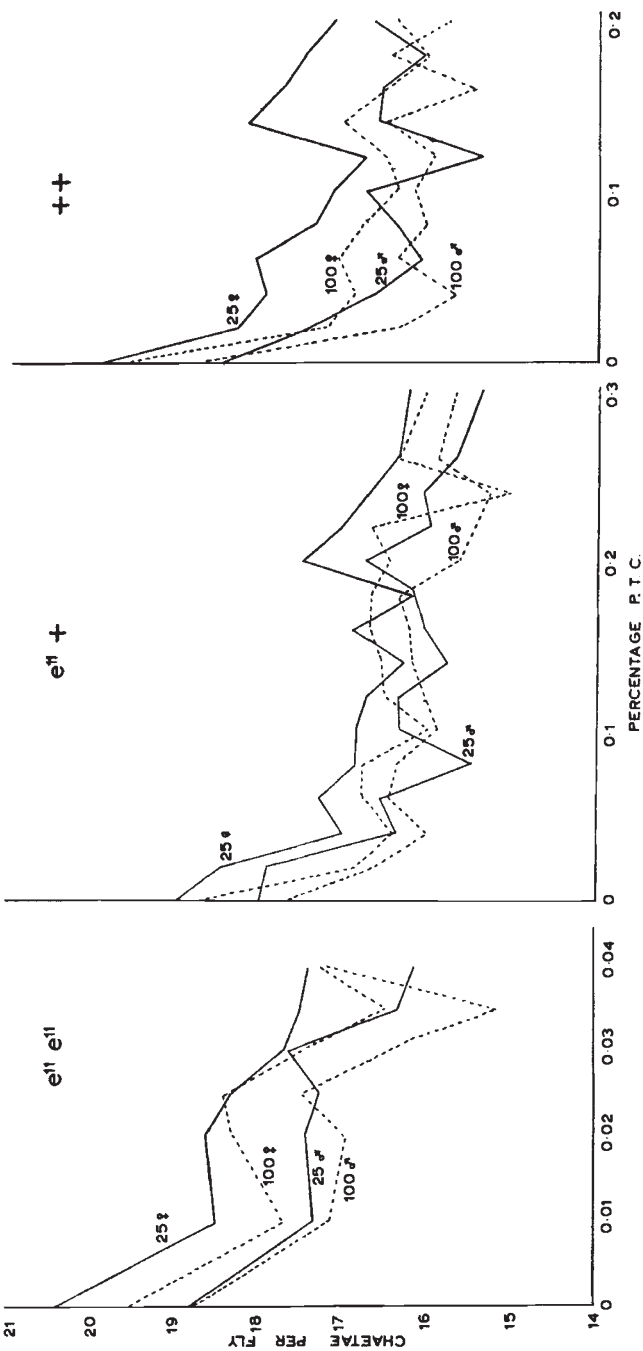

FIG. 2.—Mean chacta number at different concentrations of P.T.C. for the genotypes $e''e''$, $e''+$, and $++$.

in small flies there are often 2, and sometimes it is hard to distinguish macro- and micro-chaetæ. For this reason, it does not appear justifiable to change from the measure $\frac{A}{T}$ postulated above. Such values were estimated, and for convenience multiplied by 1000 (table 1). If all asymmetry values are assumed to be equally informative, analyses of variance can be done on them. No significant effect of genotype, sex, competition, or P.T.C. concentration was apparent on asymmetry. Thus the stress of P.T.C. which increased fly weight variability, did not increase chaeta number variability or asymmetry, even though there appears to be a correlation between chaeta number and fly weight. This conclusion is perhaps a little surprising, but may be resolvable if we can determine more precisely when chaeta numbers and positions are determined in the developing fly, and the variability of fly size at this stage.

5. CHAETA NUMBER AND FLY WEIGHT

As a preliminary, mean chaeta number was plotted against fly weight for each sex and level of competition for each concentration of P.T.C., provided that the mean was based on more than 5 observations. In fig. 3, the resultant scatter diagrams are given for the 3 genotypes. It is obvious that chaeta number and fly weight are correlated as suggested earlier from figs. 1 and 2. Fig. 3 shows that this correlation appears to be linear, thus chaeta number is proportional to fly weight.

To confirm this, a linear regression analysis of chaeta number on fly weight must be done. Such regression coefficients, which are all highly significant ($P < 0{\cdot}001$), are given for various contrasts in table 6. Comparing the total data for the 3 genotypes, we see that the $e''+$ coefficient is less than the $++$ and $e''e''$ coefficients. In fact, a test of significance (table 6) reveals that this difference is significant. Thus chaeta number decreased relatively less rapidly with decreasing weight in the heterozygotes than in the homozygotes. We have seen that the heterozygotes survive better at higher concentrations of P.T.C. (table 4). If sterno-pleural chaeta number is of adaptive value, as is certainly the case, then it is reasonable that there is a relationship between greater survival ability of $e''+$, and a smaller rate of decrease of chaeta number with decreasing fly weight, than occurred in the homozygotes. The heterozygotes may therefore have somewhat better developmental stability than the homozygotes.

We can also test for the effects of sex and competition within each genotype. If all the regression coefficients given in table 6 (*i*) (*b*) are assumed to be equally accurate, *i.e.* contribute equal information, a simple *t* test will test the difference between sexes and levels of competition. The assumption of equal information is reasonable, since the number of degrees of freedom on which the coefficients in

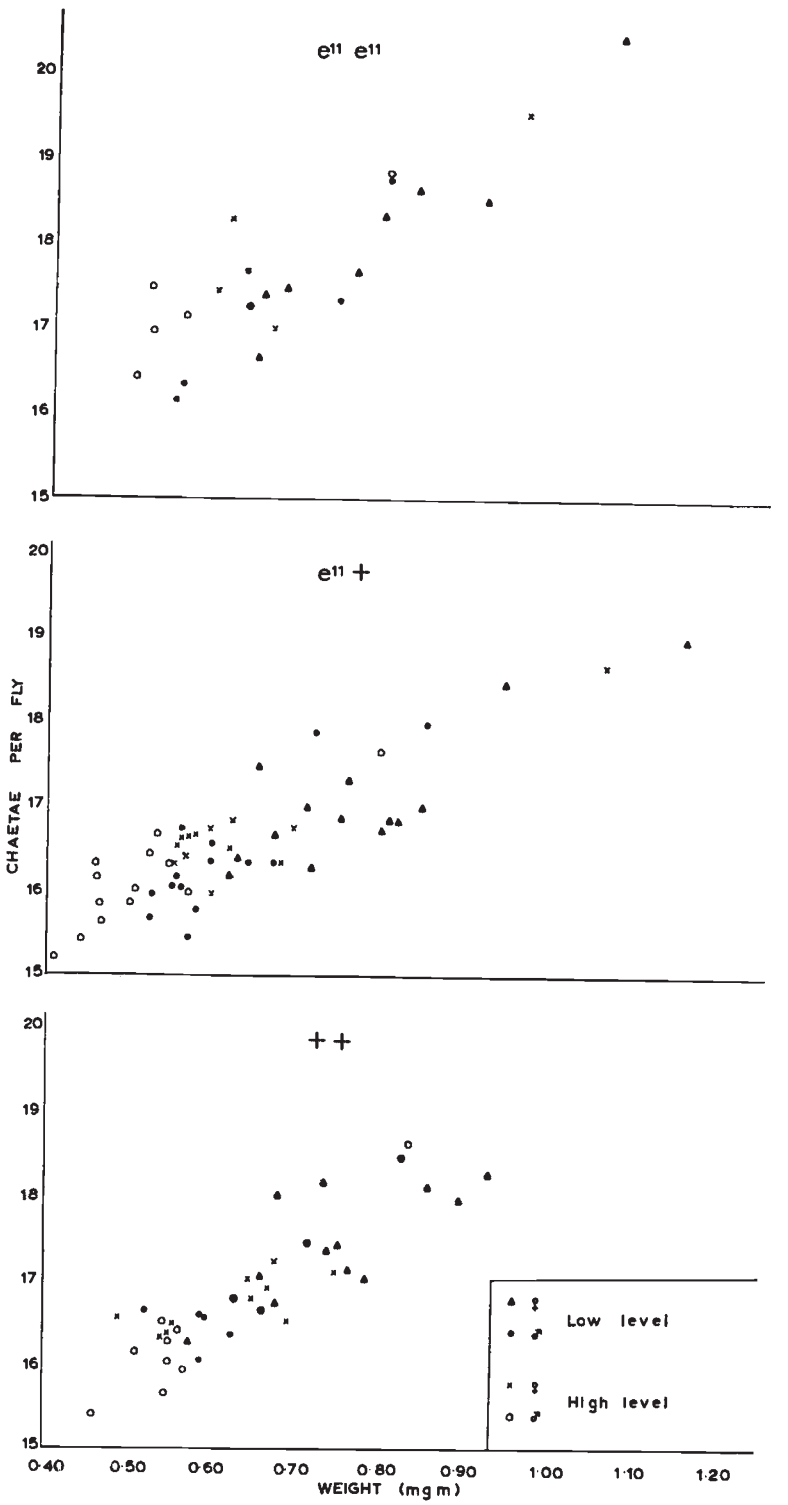

FIG. 3.—The relation between fly weight and chaeta number for the genotypes *e″e″*, *e″*+ and ++.

table 6 (*1*) (*b*) are based do not vary much within genotypes. Mean values of the regression coefficients are given in table 6 (*1*) (*c*) for sexes and levels of competition.

The mean values show that for all genotypes and levels of competition, the male regression lines are steeper than the female regression lines, and these differences are significant (table 6 (*2*)). An interpretation of this sex difference is a little difficult to find, but it may be a result of variability of female flies induced by egg formation.

TABLE 6

(1) *Regression coefficients of chaeta number on fly weight (mgm.)*

	e″e″	*e″*+	++
(*a*) Total data	0·053	0·042	0·057
(*b*) Low level of competition			
Females	0·050	0·043	0·052
Males	0·066	0·060	0·059
High level of competition			
Females	0·042	0·040	0·053
Males	0·069	0·053	0·073
(*c*) Totals (competition)			
Low	0·058	0·051	0·056
High	0·055	0·046	0·063
Totals (sexes)			
Females	0·046	0·041	0·053
Males	0·067	0·056	0·066

All regression coefficients are significantly $>0 (P<<0{\cdot}001)$.

(2) t *tests on the regression coefficients in table 6* (*1*) c

	e″e″	*e″*+	++
Low *v.* High level of competition . . .	$0{\cdot}7<P<0{\cdot}8$	$0{\cdot}2<P<0{\cdot}3$	$0{\cdot}1<P<0{\cdot}2$
Females *v.* Males	$P<0{\cdot}02$	$P<0{\cdot}001$	$P<0{\cdot}01$

Total data	*e″e″* v. *e″*+	$P<0{\cdot}02$
	e″e″ v. ++	$0{\cdot}2<P<0{\cdot}3$
	e″+ v. ++	$P<0{\cdot}001$.

The level of competition has no effect on the slope of the regression lines. Presumably, as shown in figs. 1 and 2, the high level of competition leads to smaller flies, with proportionately fewer chaetæ, but the rate of decrease of fly weight with chaeta number does not vary between levels of competition. It appears therefore that a competition effect found by Gibson (unpublished) is related to fly size. He found that flies grown in 4-inch vials had fewer sterno-pleural chaetæ than those grown in half-pint milk bottles, presumably because competition is more intense in vials so giving smaller flies. Furthermore, Rasmuson (1952) found that flies had fewer abdominal and sterno-pleural chaetæ at a high level of competition.

6. FLY WEIGHT, TEMPERATURE AND CHAETA NUMBER

Firstly, we will consider the effect of temperature on the control medium (table 7). The following conclusions are apparent; (1) fly weight was reduced by increased temperature, (2) chaeta number was reduced by increased temperature as found in previous experiments (see Rasmuson, 1952, and Thoday, 1958), (3) fly weight and chaeta number were lower at the high level of competition at a given temperature than at the low level, (4) at the low level of competition, coefficients of variation of fly weight did not differ appreciably between temperatures, but at the high level, the variability of fly weight was

TABLE 7

Fly weight and chaeta numbers at 25° C. and 30° C.

	Fly weight (mgm. × 100)		Fly weight coefficient of variation		Chaeta no.		Chaeta no. coefficient of variation		Chaeta no. asymmetry $\left(\frac{A}{T} \times 1000\right)$	
25° C.	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control (Low) *	111·38	84·98	0·077	0·091	19·90	19·16	0·075	0·086	60	60
Control (High) †	88·38	66·55	0·111	0·090	17·87	17·17	0·077	0·079	51	50
0·02 per cent. P.T.C.†	60·15	49·73	0·189	0·220	16·89	16·25	0·086	0·083	37	59
0·04 per cent. P.T.C.†	54·58	45·44	0·206	0·196	17·05	16·02	0·092	0·068	53	64
30° C.										
Control (Low) *	107·61	80·07	0·074	0·087	18·98	18·07	0·091	0·092	72	45
Control (High) †	79·97	60·62	0·127	0·128	16·82	16·13	0·093	0·091	69	55
0·02 per cent. P.T.C.†	65·81	54·53	0·206	0·176	16·86	15·49	0·092	0·091	62	56
0·04 per cent. P.T.C.†	58·44	49·27	0·224	0·188	16·44	15·27	0·092	0·078	68	48

* Four replicates of 25 larvæ.
† One replicate of 150 larvæ.

greater at 30° C. than at 25° C., and (5) at 25° C. the coefficients of variation of chaeta number were lower than at 30° C.

Thus at 30° C. the flies were smaller than at 25° C., and correlated with this was a reduction in chaeta number. Variations in sternopleural chaeta number reported by other authors as due to temperature, and level of competition, therefore appear to depend at least partly on fly size. Preliminary results reported by Gibson, Parsons and Spickett (1961) confirm this.

Fly weight variability appears little affected by temperature, which is in contrast with P.T.C., where even a very low concentration of P.T.C. was effective in increasing fly weight variability. The combination of high temperature and high competition increased variability somewhat, but not to the same extent as P.T.C. in the medium. A temperature of 30° C. is a severe stress, for at 31° C. it is unlikely that more than a few flies would survive. Chaeta number variability was, however, considerably greater at 30° C. than at 25° C., whereas

P.T.C. has been shown not to affect chaeta number variability appreciably.

Data were also collected at the high level of competition on P.T.C., for each temperature. Fig. 4 shows that as expected P.T.C. reduced fly weight, but less for the 30° C. data than for the 25° C. data. Such an interaction, if significant, is a little difficult to explain, but appears to be some sort of complex genotype × environment interaction. Chaeta number was reduced by the addition of P.T.C. as expected, but the trends are not so obvious as for fly weight.

As expected, fly weight variability was increased considerably as a result of P.T.C. treatment. To find out the major cause of the

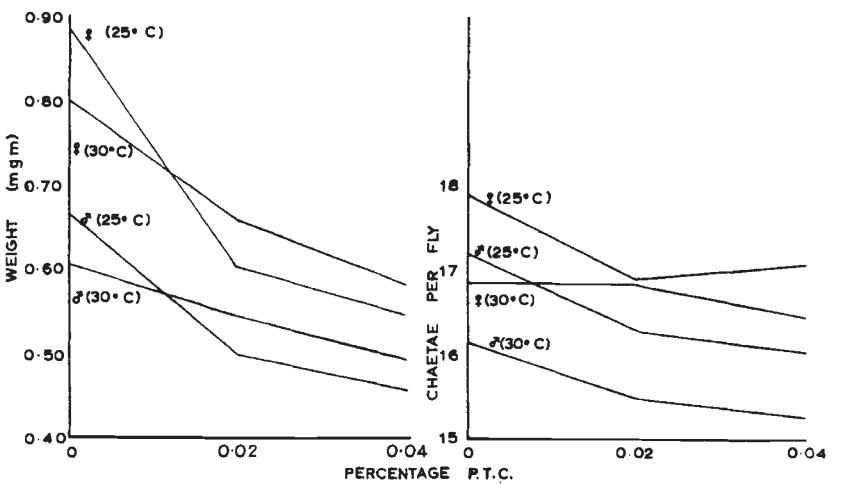

FIG. 4.—Mean fly weight and chaeta number at various concentrations of P.T.C. for the two temperatures 25° C. and 30° C.

variability of the coefficients of variation, we can do an analysis of variance to test the effect of the different treatments and sexes (table 8), assuming, as before, that the coefficients of variation are equally informative. The data collected on 0·04 per cent. P.T.C. were ignored, since very few flies emerged at 30° C. The competition and P.T.C. component is significant, showing that the variability in these data differs with level of competition and P.T.C. treatment. Although, as pointed out, the data appear more variable at 30° C. than 25° C., this increase in variability is not significant in an analysis of variance.

A high level of competition with P.T.C. present increased emergence time and its variability (table 9) as expected from the results already presented (table 5). At 30° C. emergence time was less than at 25° C., especially on 0·02 per cent. P.T.C. Thus correlated with a smaller fly at 30° C., and fewer chaetæ was a reduced development time.

Chaeta number variability was not affected by the presence of P.T.C., in agreement with previous results (table 1). In table 8 (*b*) an analysis of variance of the coefficients of variation confirms that at

TABLE 8

Analyses of variance of the coefficients of variation of fly weight and chaeta number, and of chaeta asymmetry (from data presented in table 7)

		M.S.	P
(a) Analysis of variance of the coefficients of variation of fly weight			
Competition and P.T.C.	2	14241·59	P<0·001
Sex	1	5·33	P>0·20
Temperature	1	33·33	P>0·20
Temperature × Sex	1	133·33	P>0·20
Error	6	347·58	...
(b) Analysis of variance of the coefficients of variation of chaeta number			
Competition and P.T.C.	2	9·33	P>0·20
Sex	1	5·33	P>0·20
Temperature	1	341·33	P<0·01
Temperature × Sex	1	12·00	P>0·20
Error	6	12·89	...
(c) Analysis of variance of chaeta asymmetry (measured as A/T × 1000)			
Competition and P.T.C.	2	33·08	P>0·20
Sex	1	56·33	P>0·20
Temperature	1	147·00	P<0·20
Temperature × Sex	1	385·33	P<0·10
Error	6	64·97	...

TABLE 9

Emergence time in days and its variability at 25° C. and 30° C.

	25° C. mean	Coefficient of variation	30° C. mean	Coefficient of variation	Significance of difference between means
Control (low)	7·20	0·059	6·86	0·056	P<0·001
Control (high)	9·07	0·098	8·77	0·074	P<0·01
0·02 per cent. P.T.C.	10·58	0·173	9·47	0·108	P<0·001

30° C. chaeta number variability was greater than at 25° C. At 30° C. the mean coefficient of variation of chaeta number was 0·092 and at 25° C. it was 0·081.

Finally, chaeta asymmetry was calculated as $\frac{A}{T} \times 1000$. At 30° C. the mean value of $\frac{A}{T} \times 1000 = 59{\cdot}8$, and at 25° C. it came to 52·8,

assuming all asymmetry values to be equally informative (omitting the data for 0·04 per cent. P.T.C.). The analysis of variance of the asymmetry values (table 8*b*) is suggestive of a temperature effect, but more data are needed to confirm this although greater asymmetry at 30° C. agrees with Thoday's (1955 and 1958) results. There is a suggestion of a temperature × sex interaction, which if valid, is a little difficult to interpret.

Thus, at 30° C., flies deteriorate in developmental homeostasis as a result of the environmental change; accidents of development being more frequent at this temperature. It was noted, too, that out of 315 flies classified at 30° C., 10 had crumpled or curled wings, whereas at 25° C. out of 451 classified, none was affected. One fly at 30° C. was exceptional in having 24 sterno-pleural chaetæ on the left side and 9 on the right side. This fly was so grossly abnormal that it was omitted from the main analysis. These abnormalities merely confirm that at 30° C. developmental homeostasis breaks down fairly generally.

7. DISCUSSION

(i) *Variability of fly weight and chaeta number*

In this paper we are studying the effect of three environmental stresses, namely P.T.C., which is known to reduce fly size, temperature which is known to affect chaeta number, and competition. The results indicate a fairly direct correlation between fly size as measured by weight, and chaeta number for these three stresses.

The environmental stresses, P.T.C. and temperature, differ in nature. P.T.C. reduced fly weight and increased its variability. Emergence time was delayed by P.T.C., and its variability increased. P.T.C., however, had little effect on chaeta number variability and asymmetry. It therefore has little effect on the developmental stability of flies, and constitutes a stress affecting fly weight, but not the variability of the morphological structure of the fly.

Ebony larvæ die on lower concentrations of P.T.C. than wild-type larvæ (Kroman and Parsons, 1960), since the tyrosinase activity of ebony larvæ is lower (Ohnishi, 1954). Correlated with this is an increase of the size of the nuclei of the corpus allatum portion of Weismann's ring in ebony larvæ (Wolsky and Kalicki, 1959). Perhaps the somewhat longer development time of ebony larvæ (table 5) is connected with some sort of variation in juvenile hormone synthesis from the larger, and presumably more polytenic, nuclei of ebony larvæ, and this leads, as suggested by Wolsky and Kalicki (1959) to an eventual inhibition of tyrosinase activity. P.T.C., by inhibiting tyrosinase, may have the effect of delaying pupation by a complex interaction with the juvenile, or related, hormones. It is unlikely that such a mechanism would also increase chaeta number variability and asymmetry appreciably, whereas it seems reasonable that fly weight and emergence time are much more susceptible to P.T.C., since these are the components of fitness more directly under stress.

Temperature, however, increased chaeta number variability and asymmetry at 30° C. because of poorer developmental stability of the morphological structure of the fly, whereas fly weight and emergence time variability were little affected. In this case, the component of fitness which is reacting most to the stress by increasing its variability is chaeta number. Furthermore, other abnormalities were common, such as crumpled and curled wings. Although fly weight was reduced slightly, this was obviously not affected critically, and presumably at temperatures a little above 30° C., death would occur because of general poor homeostasis of the morphological structure of the fly.

P.T.C. specifically affects tyrosinase production and is therefore a much more " specific " environmental stress than temperature which probably affects many developmental pathways differentially so that various interactions would occur at different temperatures. Temperature is therefore a " general " environmental stress.

TABLE 10

A comparison of flies at 17° C. and 25° C. (150 larvæ per replicate)

	Weight (mgm. × 100)		Chaeta no.		Emergence time (days)	
	♀	♂	♀	♂	♀	♂
17° C. . .	98·57	81·56	19·87	18·95	16·96	17·05
25° C. . .	95·83	72·63	18·85	17·81	8·69	8·57

At 30° C. flies were smaller with fewer chaetæ and developed more rapidly than at 25° C., hence, at lower temperatures we would expect larger flies with more chaetæ. Observations by Rasmuson (1952), Thoday (1958), Beardmore (1960) and Plunkett (1926) show that more chaetæ are present at lower temperatures, and a small trial at about 17° C. recently carried out (table 10) confirmed that at lower temperatures an increase in fly weight was correlated with an increase in chaeta number and a longer emergence time (see also Gibson, Parsons and Spickett, 1961). Plunkett (1926) found that the chaeta number of flies was decreased progressively by growing larvæ for increasing periods at a high temperature after transferring from a lower temperature. Hence, no specific time during larval development is involved. We are dealing with a continuous process whereby fly size and chaeta number depend on the time of exposure to various temperatures. Auerbach (1936) showed that the two sterno-pleural discs separate during embryonic development; consequently the size of the discs will be affected by larval size, so influencing the size of the sterno-pleural plate and chaeta number.

On these arguments, in a line selected for high chaeta number, flies would be larger, and perhaps take longer to develop. It seems

reasonable that as selection proceeds interactions would occur, and the regression of chaeta number on fly size would become non-linear, such that chaeta number would increase relatively more than fly size since chaeta number is being selected directly. Preliminary results confirming this (Gibson, Parsons and Spickett, 1961) indicate that fly size as measured by weight and by the surface area of the sterno-pleural plate, is increased after selection for sterno-pleural chaeta number; but the increase in fly size is less than would be expected on the basis of the regressions presented in this paper. It seems worth while recording fly size and development time as concomitant observations during the progress of an experiment in which chaeta number is being selected. Non-linearity would be expected in the phenomenon of accelerated response to selection (Sismanidis, 1942; Mather and Harrison, 1949;

TABLE 11

	++		*e*″+	
	Chaetæ	Weight (mgm. × 100)	Chaetæ	Weight (mgm. × 100)
Control (25 larvæ per replicate)	18·42	82·13	17·99	84·88
0·16 per cent. P.T.C. (100 larvæ per replicate)	15·41	45·95	16·17	45·43
Per cent. reduction .	16·34	44·05	10·12	46·48

Thoday and Boam, 1961), since this is due to the occurrence of recombinants for high chaeta number between interacting polygenes. The recombinants are then favoured by selection, and so chaeta number would rise rapidly, whereas fly size would hardly be expected to be affected so radically.

(ii) *Chaeta number and fly size*

Reeve (1960) reported that a reduction of 23 per cent. in thoracic area was accompanied by a reduction in sterno-pleural chaeta number of 18·5 per cent. in the mid-parent between two Pacific inbred lines and 13·1 per cent. in the F_1. Comparing ++ and *e*″+ we get, for example, the results shown in table 11.

Hence the per cent. weight reduction is greater than the per cent. chaeta reduction, and on the basis of Reeve's data, it is also greater than the surface area reduction, since about the same chaeta reduction (< 20 per cent.) is accompanied by about 45 per cent. weight reduction and 23 per cent. thoracic area reduction. In small flies, therefore, chaetæ are more crowded than in large flies, and this occurs for sternite chaetæ (Robertson and Reeve, 1954). It seems legitimate to conclude

that chaeta number falls with decreasing surface area and volume but less rapidly than both.

Robertson and Reeve (1954) found that females had 20 per cent. more abdominal sternite chaetæ than males, and on the basis of the linear dimensions of the flies, they concluded that sternite chaeta density is the same in both sexes. The sex-difference for sterno-pleural chaetæ, however, appears to be less; the females having 5-10 per cent. more chaetæ than the males in the data presented in this paper. The presence of 3 macro-chaetæ on each side in most flies indicates a greater degree of differentiation in the sterno-pleural plate than in the sternites. The macro-chaetæ, although occasionally absent in smaller flies, vary less than the micro-chaetæ, perhaps because they are of greater adaptive significance. This may have the effect of making the sex-difference for sterno-pleural chaeta number less than for sternite chaeta number. In general, it may be expected that the more closely related to fitness a morphological structure is, the less variable it will be.

8. SUMMARY

1. Larvæ of three genotypes, ebony, wild-type and heterozygous ebony were grown on various concentrations of phenyl-thio-carbamide (P.T.C.) at two levels of competition, namely 25 and 100 larvæ per replicate. In general, as P.T.C. concentration increased, fly size decreased, and fly size variability increased. Emergence time and its variability both increased with increasing P.T.C. concentration. Sterno-pleural chaeta number decreased, but its variability was unaffected, which suggested that P.T.C. did not affect the developmental stability of the components of the fly, but only the size of the fly, perhaps by an interference with hormone production.

2. At the high level of competition, flies were smaller, had fewer chaetæ, and took longer to emerge than at the low level, but fly weight variance and chaeta number variance were not affected, although emergence time variance increased, no doubt in response to the additional stress involved.

3. The linear regression of fly weight on chaeta number was significant for all genotypes, but the regression line for the heterozygotes was significantly less steep than for the homozygotes, perhaps due to better homeostasis of the heterozygotes. A sex difference in regression lines was found and is perhaps due to the greater variability of females with developing eggs.

4. As the flies became smaller, the chaetæ became more crowded, even though there was a reduction in chaeta number. The percentage reduction of weight was greater than the percentage surface area reduction which was greater than the percentage chaeta number reduction, when comparing different fly sizes.

5. Flies grown at 30° C. were smaller, developed more rapidly, and had fewer chaetæ than those grown at 25° C. Fly weight, and

emergence time variability were not affected appreciably at 30° C., but sterno-pleural chaeta variability and asymmetry were increased, suggesting that temperature is a more "general" stress than P.T.C. for it affects the developmental stability of the components of the fly. The temperature data also indicate that fly size is in general correlated with chaeta number and emergence time. Hence, if in a selection experiment, chaeta number is being selected, it is plausible that there may be a partially correlated response of emergence time and fly size.

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