

Genetic and environmental control of a continuous trait, *Abnormal abdomen*, in *Drosophila melanogaster*

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SUMMARY

The mutant phenotype *Abnormal abdomen* is under the control of a major gene, A^{53g} , located distally on the X chromosome. The phenotypic abnormalities are a result of the developmental interaction between this major gene and a modifier system associated with the residual genotype. The primary developmental effect of this mutant genotype is an interference with adult histoblast differentiation resulting in a raggedness or loss of tergite material due to the changes in the formation of the adult abdominal hypoderm. A secondary effect is an interference with middorsal fusion of the histoblasts.

The developmental effect of the genotype is influenced by a number of factors which include crowding, humidity, temperature, and the age of the culture. The mutant phenotype is due to an interaction of the major gene and the modifier system in association with these environmental factors. Gene action in relation to the final phenotype is postulated to be a two-step affair. The first is an increase in protein synthesis under the influence of the modifier system; and the second is the reaction of the histoblast, under the influence of the major gene, to this increase in protein synthesis during its differentiation into adult hypoderm. The function of the environment in this sequence of developmental reactions is postulated to be in its control of the utilization of the increased protein associated with the action of the enhancer genes. This hypothesis is discussed in terms of (1) the production and utilization of gene products and of (2) the regulation of development in terms of this production and utilization in a balanced developmental system.

1. INTRODUCTION

The majority of mutant genes studied in the metazoa and metaphyta have an expression which is comparable to an all-or-none effect on the phenotype. The presence or absence of the mutant gene can be diagnosed immediately by a sudden, discontinuous change in the phenotype of the organism. There are, however, a number of mutant genes whose penetrance and expressivity range from an extreme change in the appearance of 100% of the affected individuals to a small phenotypic change in a low percentage of the organisms in which this gene is found. This range of penetrance and expressivity has been attributed to various factors which may influence gene action in multicellular organisms. Among these are

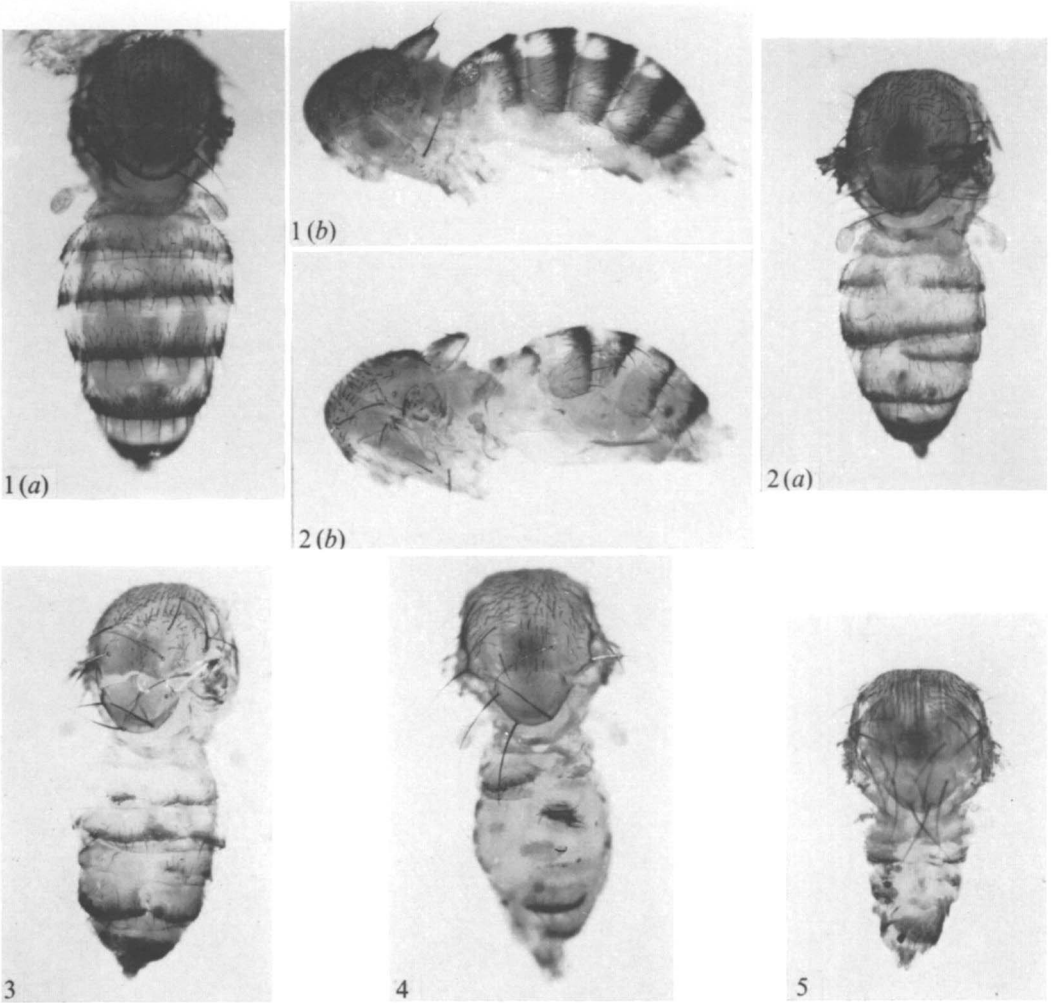
environment, modifier genes, and heterochromatic position effects. The physiological control of the penetrance and expression of these genes has been attributed by several workers, mainly Goldschmidt (1938, 1956), Landauer (1957) and more recently Sang (1963) and Rendel (1962), to a threshold phenomenon which explains the switch to alternate developmental pathways as being due to the interaction of the gene with the total physiological milieu of the developing organism.

This communication is a description of an inherited abdominal abnormality in *Drosophila*. The mutant phenotype is one which has been reported in a number of inbred lines of *Drosophila* and which has always been found to behave in a complicated way both genetically and developmentally. At least three such cases have been investigated. Morgan (1911, 1915) described a sex-linked mutation affecting tergite formation, the expression of which was under the control of the environment; Sobels (1952) reported a polygenically inherited abdominal abnormality, *Asymmetric*, which was controlled by both genetic and environmental factors; and Kobel & van den Bosch (1970) described the developmental genetics of an autosomal recessive gene in *Drosophila hydei* which affected the formation of sternites, and secondarily tergites, during metamorphosis. This report is an attempt to explain the variable phenotypic effects known to be associated with mutations affecting the *Drosophila* abdomen on the basis of interactions between the genotype controlling the development of the tergites and the environmental conditions under which this genotype must act.

2. MATERIALS AND METHODS

The mutant stock used in these experiments was derived from a sex-linked lethal stock which carried the 'Muller-5', $sc^{S1}BInSc^8$ marked inversion X chromosome as a balancer. The lethal-bearing chromosome originated in an inbred *Canton-S* stock which had been exposed to X-irradiation, and the abdominal abnormalities reported here appeared during the localization of the lethal mutation. The factor responsible for the abdominal changes was separated from the lethal factor by recombination and reintroduced into the *Canton-S* genetic background. This line was then designated as *Abnormal abdomen* (A^{53g}) and selected for the mutant phenotype. The stock has been selected and inbred for several hundred generations and presently shows a stable penetrance and expressivity for the mutation.

The method of calculation of penetrance and expressivity of the mutant gene is based upon the original definition of these terms by Timoféeff-Ressovsky (1931). Penetrance is calculated as the frequency of flies which show any degree of abdominal abnormality among those flies known to carry A^{53g} . This, therefore, includes any degree of expression of the mutant phenotype. Expressivity is calculated by assigning a grade of zero (0) to the wild-type phenotype, one (1) to the slightly abnormal abdomens, two (2) to the intermediate abnormalities, and three (3) to those flies with extremely abnormal abdomens. These phenotypes are described below. A mean expressivity for all individuals carrying A^{53g} was then



Figs. 1. Dorsal and lateral views of normal tergite structure on phenotypically wild-type female abdomen (expressivity grade 0).

Figs. 2. Dorsal and lateral views of grade 1, slightly abnormal females. Animals have less than one tergite missing.

Fig. 3. Dorsal view of grade 2 female abdomen. More than one but less than three tergites are absent.

Fig. 4. Dorsal view of a grade 3 female abdomen. Three or more tergites are absent or are present only as scattered patches of thickened cuticle.

Fig. 5. Dorsal view of a grade 3 female with a shrunken abdomen. There is incomplete formation of the abdominal hypoderm.

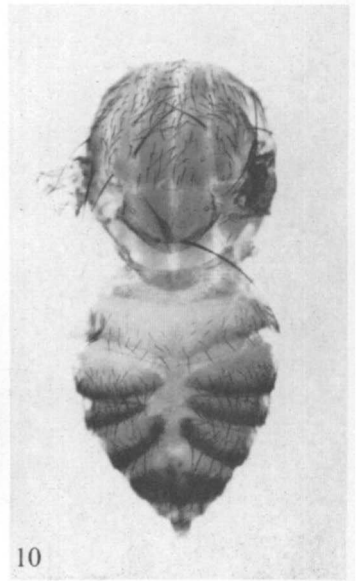
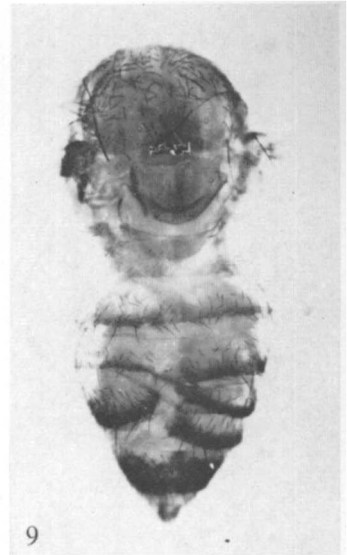
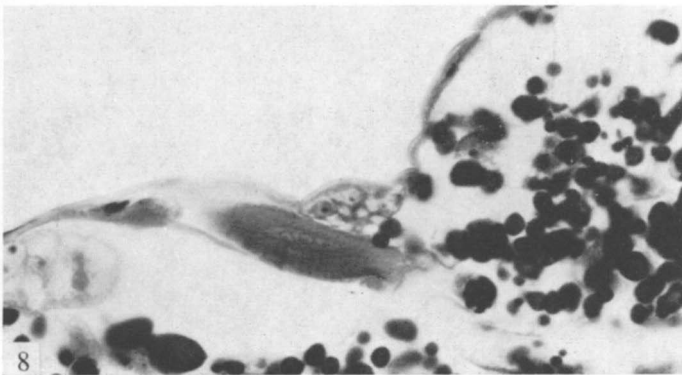
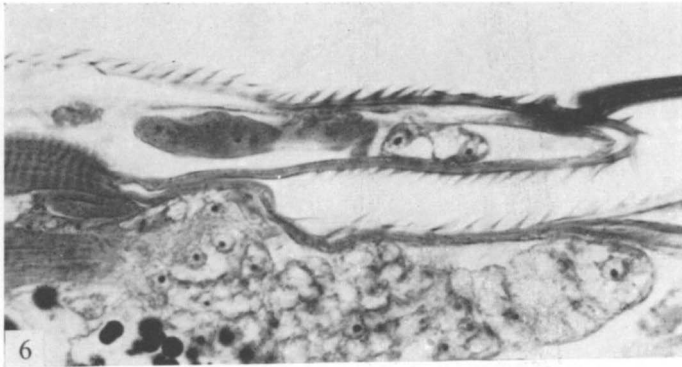


Fig. 6. Longitudinal section through an abdominal tergite of a wild-type (A^{530+}/A^{530+}) female fly. A deep fold is present and thick chitin with microchaetae present can be seen on both the dorsal and ventral surfaces of the fold.

Fig. 7. Longitudinal section of an abdominal tergite from a phenotypically wild-type, genotypically heterozygous (A^{530+}/A^{530}) female fly. The fold is shallow and the amount of hypoderm is reduced.

Fig. 8. Longitudinal section through the abdomen of a grade 3 homozygous mutant (A^{530}/A^{530}) female. Tergites were completely absent from this fly. Segmental muscles are present, but hypodermal folds are not seen.

Figs. 9, 10. Incomplete and irregular segmental fusion on the dorsal surface of homozygous A^{530} females. This phenotype is typical of *Asymmetric* but is only rarely found in the A^{530} inbred stock.

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calculated for each experiment using a simple frequency distribution ranging from 1 to 3. In this way the experimental results showing a higher mean expressivity indicate a more extreme expression of the phenotypic abnormality.

All stocks were maintained and all experiments were done using standard *Drosophila* culture techniques on a cornmeal-karo agar medium seeded with live yeast. Experiments were at 25 °C unless otherwise noted.

3. RESULTS AND CONCLUSIONS

(i) *Description of the mutant phenotype*

The phenotypic expression of the mutation, A^{53g} , involves a loss of tergite material which begins laterally on the abdomen and proceeds dorsally as the expression becomes more extreme. As indicated in Plate 1, figures 2–4, the abnormality itself can be highly variable. In the most extreme cases, Plate 1, figs. 4 and 5, there is almost complete loss of the tergite material, with only small patches of the thickened hard cuticle remaining on the dorsal abdomen. In these extreme cases the abdomen is often shrunken as well as deformed, suggesting a reduction in the amount of abdominal hypoderm in the adult.

In longitudinal section, Plate 2, fig. 6, the dorsal surface of the wild-type abdomen is composed of a folded hypodermal layer. The thick chitinous plates are produced on the exposed surfaces. In longitudinal sections of mutant abdomens this folding is greatly reduced (Plate 2, fig. 7) or completely absent (Plate 2, fig. 8) and the plates do not form. These histological observations indicate that the primary genetic effect is on the formation of the hypodermal layer itself, while the gross morphological abnormality is a secondary effect traceable to the abnormal development of the hypodermal cells.

This conclusion is supported by a second type of abnormality, similar to one reported by Sobels (1952), and occurring with low frequency in this abnormal stock. In this case there is an abnormal fusion of the hypodermal imaginal disks so that the tergites are not segmentally arranged. The result is asegmental fusion at the dorsal midline (Plate 2, fig. 9). In several instances it was noted that the tergites remained unfused at the dorsal midline (Plate 2, fig. 10). Serial longitudinal sections of these abdomens showed a lack of folding in the abnormal region and support the hypothesis that the imaginal hypoderm is primarily affected.

This absence of dorsal fusion, together with an asegmental banding pattern, was observed in the larval stages as well as the adult. In the case described here, however, the unfused, asegmental phenotype appears only infrequently. The majority of the abnormal cases involve a lack of formation of the tergite itself. Therefore the ragged appearance of the plates is the major phenotypic effect of the mutant genotype. The loss of larval cuticular material, as expressed by a raggedness of segmentation and a patchy appearance of the puparium, was never observed. The difference in phenotypic effect between the case reported here and that reported by Sobels (1952), supported by the genetic data reported below, indicates that there are at least two different genetic controls of tergite formation.

A number of factors, both genetic and environmental, can be shown to affect the observed variability in the penetrance and expressivity of A^{53g} . Homozygous A^{53g} females were mated to males from a number of inbred laboratory stocks. Development then took place under identical environmental conditions and progeny were scored for their abdominal abnormalities. The range of penetrance

Table 1. Penetrance in males carrying the A^{53g} X chromosome and heterozygous for autosomes from various inbred stocks

Origin of autosomes	No. of males	No. of abnormal males	Penetrance
Oregon-R	205	190	0.93 ± 0.02
Canton-S	136	110	0.81 ± 0.03
<i>scgccvct⁶vg²f</i>	245	64	0.26 ± 0.03
<i>yeccvfcar</i>	307	91	0.30 ± 0.03
<i>yw</i>	1019	134	0.13 ± 0.01
<i>wec</i>	579	272	0.47 ± 0.02
<i>aldpb pr Blcp_{xsp}</i>	153	124	0.81 ± 0.03
<i>ruhth stcu sr e⁸ca</i>	130	60	0.46 ± 0.04
<i>Sb In(3LR)Ubx¹⁰¹</i>	127	10	0.08 ± 0.02

Table 2. Change in penetrance and expressivity correlated with day of emergence in the inbred stock of A^{53g}

Day of emergence	Females			Males		
	Total flies	Penetrance	Mean expressivity	Total flies	Penetrance	Mean expressivity
1	60	1.00	2.80 ± 0.05	93	1.00	1.56 ± 0.06
2	113	1.00	2.91 ± 0.03	178	1.00	1.86 ± 0.05
3	238	1.00	2.87 ± 0.02	245	1.00	1.75 ± 0.04
4	256	1.00	2.86 ± 0.02	231	1.00	1.61 ± 0.04
5	289	1.00	2.68 ± 0.03	240	0.98 ± 0.01	1.55 ± 0.04
6	295	1.00	2.51 ± 0.04	203	0.97 ± 0.01	1.27 ± 0.03
7	297	1.00	2.19 ± 0.04	160	0.93 ± 0.02	1.19 ± 0.04
8	266	0.98 ± 0.01	2.02 ± 0.05	120	0.84 ± 0.03	1.07 ± 0.02
9	196	0.97 ± 0.01	1.67 ± 0.06	104	0.84 ± 0.04	1.06 ± 0.02
10	194	0.95 ± 0.02	1.39 ± 0.04	78	0.79 ± 0.05	1.02 ± 0.02
11	143	0.91 ± 0.02	1.28 ± 0.05	59	0.83 ± 0.05	1.00 ± 0.00
Total	2347	0.98 ± 0.002	2.31 ± 0.02	1711	0.95 ± 0.005	1.46 ± 0.01

of the mutant genotype in hemizygous male progeny containing a variety of heterozygous genotypes (Table 1) is evidence for an extensive influence of the residual genotype on the penetrance of this mutation. The penetrance in the presence of different heterozygous autosomes ranged from frequencies of 8% to 93%.

The effect of the environment on the expression of A^{53g} can be seen in Table 2. Comparisons of the extent of the abnormality in either females or males from the inbred mutant stock during the period of emergence in a culture bottle show that the expressivity in flies emerging from older cultures is lower than that in earlier emerging flies. A phenomenon similar to that reported here was described by

Morgan (1915) in his discussion of the role of the environment on the determination of the phenotype of *Abnormal* (*A*). He ascribed a lowered penetrance in older culture bottles to a drying out of the food as the bottle aged and as the larvae aerated the media. In the case reported here low humidity had a slight effect on the penetrance of the abdominal abnormalities and a significant effect on the expressivity of the mutation (Table 3). Those flies which developed at the higher relative humidity showed a significantly greater expression of the abnormal phenotype.

Table 3. *Penetrance and expressivity in females of the mutant genotype under conditions of high or low relative humidity*

(*A*^{53g} cultures were placed in closed chambers containing either water or anhydrous CaSO₄.)

	No. of flies	Class				Penetrance	Mean expressivity
		0	1	2	3		
Low humidity	390	16	133	96	145	0.96 ± 0.01	2.03 ± 0.04
High humidity	152	2	19	33	98	0.99 ± 0.01	2.53 ± 0.06

Table 4. *Penetrance and expressivity in females of the mutant genotype under conditions of crowding*

Egg-laying ♀♀/bottle	No. of flies	Class				Penetrance	Mean expressivity
		0	1	2	3		
4	1575	21	441	477	636	0.99 ± 0.00	2.13 ± 0.02
8	2884	96	1030	1017	741	0.97 ± 0.00	1.89 ± 0.01

In addition to humidity the effect of crowding on the frequency of abnormalities was also tested. Table 4 shows the comparison of penetrance and expressivity when four and eight females were allowed to lay eggs on a measured amount of food for 8 days. The data is a compilation of eight bottles in each class and shows that there is a significant increase in expressivity associated with development under less-crowded culture conditions.

Environmental factors other than the age of the culture, humidity and the crowding effect have been reported as having an influence on the penetrance and expressivity of mutations affecting tergite formation. Both Sobels (1952) and Kobel & van den Bosch (1970) reported a loss of penetrance of gene controlled abdominal abnormalities at low temperatures. As shown in Table 5, there is a reduction in both penetrance and expressivity in *A*^{53g} when mutant flies are raised at 18 °C as opposed to 25 °C. The table also shows that the lower developmental temperature reduces the effect of the age of the culture on the penetrance of the abdominal abnormality. The penetrance and expressivity of the abnormality remain relatively constant at 18 °C while the expected decrease in penetrance and expressivity is shown at 25 °C. The possibility that the results of this experiment

might be explained by a lethal effect of the low temperature is unlikely. At 18 °C there were no extremely abnormal flies of either sex at any time during the emergence period. This, together with the observation that pupal lethality was low (less than 10%), argues against a low-temperature effect which would increase the abnormality to the point where it becomes lethal. The loss of the ageing effect together with the reduction in penetrance and expressivity are therefore both considered to be a part of the low-temperature effect modifying the gene-controlled developmental processes leading to abdominal hypoderm and tergite formation.

Table 5. *Penetrance and expressivity in A^{53g} flies over life of culture at high and low temperatures*

Day of ecdysis	18 ± 1 °C			25 ± 1 °C		
	Total flies	Penetrance	Expressivity	Total flies	Penetrance	Expressivity
1	4	0.00	—	42	1.00 ± 0.00	2.50 ± 0.10
2	55	0.42 ± 0.07	1.00	88	1.00 ± 0.00	2.49 ± 0.08
3	34	0.50 ± 0.09	1.00	39	1.00 ± 0.00	2.44 ± 0.11
4	72	0.39 ± 0.06	1.11 ± 0.06	39	0.97 ± 0.02	2.16 ± 0.14
5	40	0.42 ± 0.08	1.00	73	0.89 ± 0.01	1.88 ± 0.11
6	37	0.43 ± 0.08	1.06 ± 0.06	65	1.00 ± 0.00	1.65 ± 0.08
7	30	0.27 ± 0.08	1.00	40	0.80 ± 0.05	1.44 ± 0.10
8	30	0.47 ± 0.09	1.00	56	0.71 ± 0.06	1.10 ± 0.05
9	18	0.11 ± 0.07	1.00	65	0.69 ± 0.06	1.07 ± 0.04
10	17	0.47 ± 0.11	1.00	—	—	—
Total	337	0.40 ± 0.03	1.03 ± 0.01	507	0.90 ± 0.01	1.91 ± 0.04

Table 6. *Recombination between A^{53g} and genes located at the distal end of the X chromosome*

Genotype	Males	Normal	Abnormal
<i>A^{53g}/wec</i> female × <i>wec</i> male			
+	579	307	272
<i>wec</i>	564	557	7
<i>w</i> +	17	17	0
+ <i>ec</i>	20	9	11
<i>A^{53g}/yw</i> female × <i>yw</i> male			
+	1019	885	134
<i>yw</i>	744	744	0
<i>y</i> +	1	1	0
+ <i>w</i>	6	5	1

(ii) *Inheritance of the abnormal phenotype*

The mutant gene responsible for the phenotypic abnormality is located at the distal end of the X chromosome (Table 6). Its position has been found to correspond roughly with that of the original *Abnormal* gene first reported and described by Morgan (1911). In the original case, Morgan (1915) reported the locus to the right of *white*. Based on recombination data presented here, *A^{53g}* lies to the

left of *white*, between *yellow* and *white* at the distal end of the *X* chromosome. The mutation reported here has been described as a recurrence of the original *Abnormal* (Lindsley & Grell, 1968) for two reasons. First, the description of the mutant phenotype almost exactly parallels the description of *Abnormal*, and secondly, the penetrance and expressivity of the mutant gene depend upon environmental factors which correspond directly to those described by Morgan (1915).

Table 7. Test for sex-linked modifiers of A^{53g} using recombination between an *X* chromosome carrying the mutant major gene and a marker *X* chromosome

	Total females	Abnormal	Penetrance
<i>sc eccvct⁶vg²f</i>	92	46	0.50 ± 0.05
<i>sc eccvct⁶vg²+</i>	113	59	0.52 ± 0.05
<i>sc eccvct⁶v++</i>	132	91	0.69 ± 0.04
<i>sc eccvct⁶+++</i>	109	52	0.48 ± 0.05
<i>sc eccv++</i>	193	125	0.65 ± 0.03
<i>sc ec++++</i>	99	51	0.52 ± 0.05
<i>sc++++</i>	222	121	0.54 ± 0.03

The data previously presented show that the abnormal phenotype is controlled not only by a single sex-linked major gene but also by a number of modifier genes. The high variability of penetrance in different heterozygous genetic backgrounds precludes the action of a single modifier gene and argues for multiple modifier gene action. Where single suppressors or enhancers are involved, the localization of the modifier is relatively simple. The complexity of the genotypic situation in this case, however, can best be illustrated by reporting two experiments; the first involving modifiers on the *X* chromosome, and the second involving a study of chromosome 3.

In the first experiment inbred A^{53g} females were mated to males carrying the sex-linked markers *sc eccvct⁶vg²f*. Heterozygous F_1 females were backcrossed to males from the inbred abnormal stock. Recombination chromosomes from these heterozygous females were then isolated in the males of the F_2 backcross generation. Matings were made between females from the A^{53g} stock and these F_2 males, carrying either the non-crossover *sc eccvct⁶vg²f* chromosome or a chromosome which arose as a single crossover involving markers from the centromere end. Each class, therefore, consisted of females who were homozygous for successively longer lengths of the *X* chromosome from the original A^{53g} line. The results are seen in Table 7.

The evidence from this experiment indicates that both enhancers and suppressors of the phenotypic effect of A^{53g} are present on the *X* chromosome of the inbred stock. There are two enhancers of the tergite malformation. One of these lies in the vicinity or to the left of *garnet*, the second lies in the vicinity or to the left of *cut*. There are also two suppressors located on the inbred chromosome (or conversely, two enhancers located on the marker chromosome). One of these is in the vicinity or to the left of *vermillion* while the second is in the vicinity or to the

left of *crossveinless*. These modifiers have not been more closely localized, but it is apparent that there is a complex sex-linked modifier effect. The presence of genes which cause an increase in penetrance of A^{53g} on both the marked and unmarked X chromosomes is evidence for this conclusion.

Table 8. Comparison of the penetrance of A^{53g} in two independently selected stocks

Stock	Sex	Total flies	Abnormals	Penetrance
A^{53g}	Female	329	328	0.996
	Male	359	332	0.924
$A^{53g}wec$	Female	698	682	0.977
	Male	824	747	0.906

Table 9. Comparison of male F_1 progeny when two independently selected A^{53g} stocks are outcrossed to the *rucuca* stock

Genotype	Total males	Abnormal	Penetrance
$A^{53g} + +$ <i>rucuca</i>	265	71	0.27 ± 0.03
+			
$A^{53g}wec$ <i>rucuca</i>	130	60	0.46 ± 0.04
+			

The second experimental test for the presence of modifier genes involved a study of the modifiers on chromosome 3. The marker chromosome used in this experiment was the mutant third chromosome, *rucuca*, containing the genes *ruhthstcusre^sca* along its entire length. The markers are well spaced except for *th* (43.2) and *st* (44.0) on the left arm. Two independent A^{53g} lines were used in this experiment. The first was the inbred stock which was previously described. The second was a stock derived from this inbred line by a recombination between A^{53g} and *wec*. This latter chromosome arose from a linkage experiment and after isolation was inbred and selected independently of the original mutant stock. The penetrance in the $A^{53g}wec$ stock was essentially the same as in the unmarked stock at the time of this experiment (Table 8).

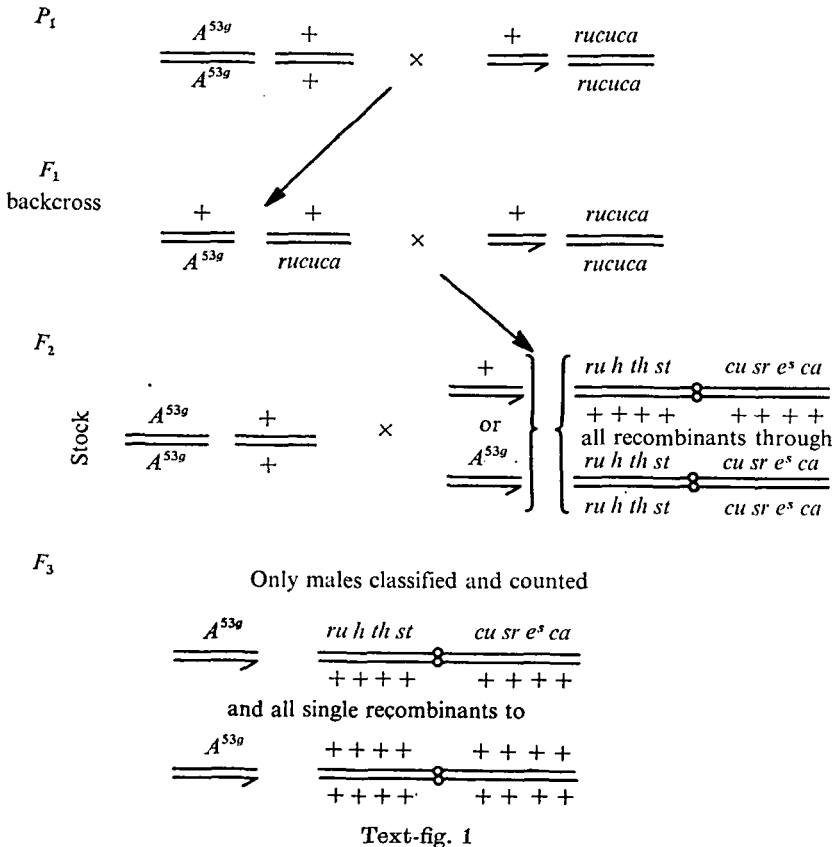
The experimental procedure involved crossing homozygous A^{53g} and $A^{53g}wec$ females to males carrying the *rucuca* markers. A comparison of male progeny from these two crosses is shown in Table 9. The data show that, although both of these inbred stocks have a similar penetrance of the abdominal mutation, the penetrance in outcrosses is quite different. Since the A^{53g} mutation in these two inbred lines is the same ($A^{53g}wec$ being derived from A^{53g} by recombination), it can be assumed that the difference in penetrance in outcrosses to *rucuca* is based upon a difference in the genetic background of the two abnormal lines. In addition, there must be modifier genes in the *rucuca* stock that interact differently with the

genetic background of the two inbred mutant lines. This latter assumption can be tested with regard to suspected modifiers on chromosome 3 by investigating the affects on penetrance after recombination of parts of the *rucuca* chromosome with the chromosome 3's of the two inbred lines.

The experimental procedure using the A^{53g} inbred stock is outlined in Text-fig. 1. The only differences between the outlined procedure and that involving the $A^{53g}wec$ stock was in the use of

$$\frac{A^{53g}wec}{A^{53g}wec} +$$

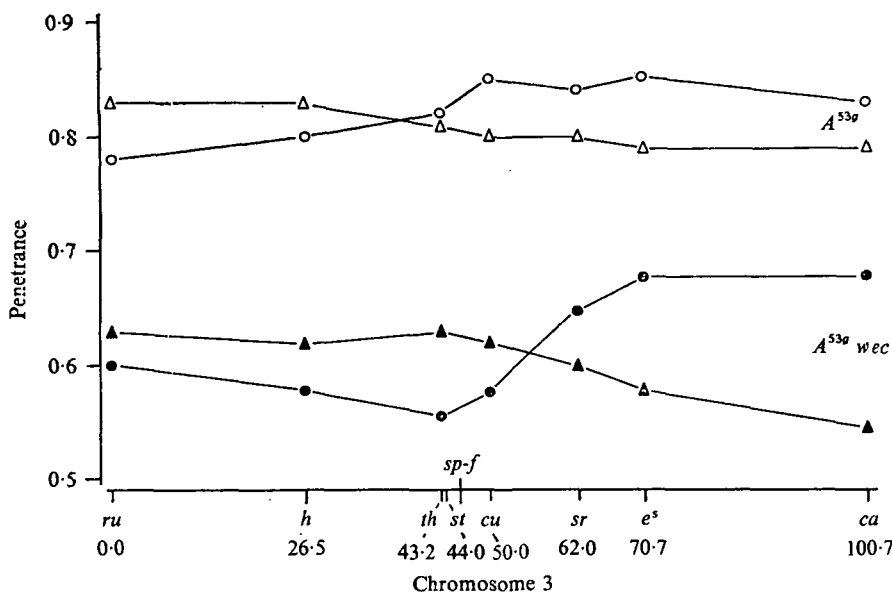
females in the P_1 generation and in the use of only $A^{53g+w^+ec^+}$ males in the F_2 . Since *white* made it impossible to distinguish both *scarlet* and *claret* either alone or



in combination, it was necessary to limit the F_2 cross to males which did not carry the $A^{53g}wec$ chromosome. However, since the X chromosomes of these males (in both crosses) were found only in the F_3 females and since only F_3 males which carried A^{53g} from their mothers were classified and counted, this difference was not considered to be critical. The F_2 females were always from the inbred A^{53g} stock

so that the comparison made was between the third chromosomes of the independently inbred mutant stocks against the third chromosome of the *rucuca* stock.

The distribution of chromosome 2 in these crosses was not controlled and was therefore a source of possible error. Data were collected, however, only from the progeny of those F_2 crosses in which at least two recombinant males were allowed to mate with four A^{53g} females. In addition, each experiment was repeated several times, and the results were consistent. The distribution of chromosome 2, therefore, should have been at random and should not have influenced the results.



Text-fig. 2. Effect of the reciprocal replacement of portions of the A^{53g} and the $A^{53g}wec$ third chromosomes by *rucuca* chromosome markers on the penetrance of abnormal abdomen. Successive replacement of the A^{53g} chromosome III by markers from the *rucuca* chromosome (O—O) and of the *rucuca* chromosome markers by their wild-type alleles from the A^{53g} chromosome III (Δ—Δ). Successive replacement of the $A^{53g}wec$ chromosome III by markers from the *rucuca* chromosome (●—●) and of the *rucuca* chromosome markers by their wild-type alleles from the $A^{53g}wec$ chromosome III (▲—▲).

Text-fig. 2 shows the results of reciprocal crosses between the *rucuca* chromosome and the third chromosomes from the A^{53g} and the $A^{53g}wec$ inbred stocks. Recombination progeny between all of the loci except *th* and *st* were observed and tested. In the $A^{53g}wec$ experiment a single recombinant between *th* and *st* was mated, and because the data indicated no difference in the two regions, the two loci were considered as one. The ordinate records the frequency of abnormal males and the abscissa records the location of the mutant loci on the *rucuca* chromosome, drawn to scale.

These experiments lead to several conclusions. The differential response of the

two inbred chromosomes to successive replacement by the *rucuca* chromosome confirms the previous observation that the two inbred lines differ in their genetic background with respect to modifiers of A^{53g} . There are modifiers on the right arm of the *rucuca* chromosome which increase the penetrance of A^{53g} in the $A^{53g}wec$ stock but have no effect in the A^{53g} stock. There are modifiers on the left arm of the *rucuca* chromosome which are opposite in their effect. Since the major genes are the same in both cases, it is obvious that there are at least two enhancing modifier genes on the *rucuca* chromosome, with the third chromosomes of the inbred mutant lines each containing only a single, different one.

In addition, the evidence shows that the third chromosome enhancer genes, present on the *rucuca* chromosome, are dominant to their alleles in the inbred lines. As the third chromosome becomes heterozygous by the replacement of successive parts of the inbred wild-type chromosome with the *rucuca* chromosome, the penetrance of A^{53g} increases. The alleles (e.g. suppressor genes) on the left arm of the A^{53g} third chromosome and in the right arm of the $A^{53g}wec$ third chromosome are recessive to the enhancer genes carried in the third chromosome of the marker stock.

(iii) *Genetic control of the gene-environment interaction*

Since it had been shown that the expressivity of the phenotypic abnormality is under the control of both genetic modifiers and the environment, an investigation of the relationship between these two controlling systems was initiated. Two

Table 10. *Change in expressivity correlated with day of emergence in the A^{53g} and the $A^{53g}wec$ stocks*

Day of emergence	A^{53g}				$A^{53g}wec$			
	Total flies		Expressivity		Total flies		Expressivity	
	Female	Male	Female	Male	Female	Male	Female	Male
1	79	109	2.52	1.75	104	97	1.99	1.61
2	122	229	2.20	1.57	169	216	2.06	1.75
3	123	178	2.17	1.53	103	165	2.07	1.74
4	99	157	1.87	1.45	94	157	1.97	1.62
5	104	138	1.90	1.51	104	118	2.00	1.66
6	88	81	1.80	1.36	130	144	1.96	1.53
7	102	114	1.59	1.30	116	121	1.95	1.44
8	116	108	1.52	1.24	148	171	1.86	1.42
9	47	43	1.51	1.31	95	89	1.83	1.37
Total	880	1157	1.92	1.48	1063	1237	1.96	1.55

different modifier systems were available in the A^{53g} and the $A^{53g}wec$ inbred lines, and the expression of the abdominal abnormalities was known to be reduced in aged culture bottles. The relationship between the two inbred genotypic backgrounds and the aged culture effect on expressivity was investigated, and the results may be seen in Table 10.

In the experiment from which the data presented was obtained, the penetrance

in the two independently inbred lines remained the same throughout the collecting period. Females in both stocks showed 100 % penetrance of the mutant genotype, while males had a daily penetrance which fluctuated between 91 % and 98 %. The expressivity of the males in both inbred lines dropped as the cultures aged; and although the males from the *A^{53g}wec* stock were slightly more abnormal, the change in expressivity was comparable in both lines. In the case of the expressivity pattern of the females plotted against day of emergence, however, the two lines show marked differences. In the *A^{53g}* inbred line there is a steep decrease in expressivity beginning almost immediately and continuing until the seventh day of emergence. In the *A^{53g}wec* females this decrease in expressivity is not as drastic, and the drop is at most eight percent, compared to approximately 30 % in the *A^{53g}* line. These data support the hypothesis that the change in expressivity in older cultures is a function of the modifier genes in the genetic background and not of the *A^{53g}* gene itself.

4. DISCUSSION

Inherited morphological abnormalities of the *Drosophila* abdomen are of two types. *Asymmetric* (*Asy*) is a polygenically controlled, autosomally inherited phenotype evidenced by incomplete or aberrant fusion of the dorsal tergites (Sobels, 1952). *Abnormal abdomen* (*A^{53g}*), as reported here and first described by Morgan (1911), is a sex-linked dominant mutation resulting in losses of lateral and dorsal tergite material. Although the penetrance and expressivity of both *Asy* and *A^{53g}* are under the control of multiple gene systems, the two mutant phenotypes differ in genetic origin. For example, no single major gene responsible for *Asy* has been localized. The penetrance of *A^{53g}*, however, is dependent upon the presence of a major mutant gene, located distally on the X chromosome, as well as upon the presence of multiple enhancer genes located throughout the genome.

One similarity between the penetrance of these two mutant genotypes is the shift during emergence in a culture bottle from an abundance of abnormal flies early in the life of the culture to an increase in the frequency of normal flies as the culture ages. In *A^{53g}* this change is under the control of the complex residual genotype. Although there is no information concerning multiple gene-environment interactions in *Asy*, such regulation is not unique to *A^{53g}*. It has also been reported for *cubitus interruptus dominant* by Sharloo & Nieuwenhuijs (1964), for *tumor brown* by Sang & Burnet (1967) and for *eyegone* and *eyeless* by Hunt (1969) and Hunt & Burnet (1969).

The data which we have reported (Hillman, Shafer & Sang, 1973) detailing a direct relationship between high protein concentration and increased expressivity of *A^{53g}* together with that data which show that the residual *A^{53g}* genotype is responsible for increased tRNA aminoacylation and amino acid incorporation into cell-free *Drosophila* systems (Rose & Hillman, 1969, 1973) suggest an explanation for the modifier gene controlled environmental influences on the expression of the *A^{53g}* phenotype. If increased protein synthesis under the control of modifier genes is responsible for the observed phenotypic abnormalities, and if this increased

protein synthesis can be offset by environmentally directed metabolic stress, a direct relationship may be postulated between modifier genes, protein synthesis, environmental effects, and the phenotypic expression of A^{53g} . This hypothesis explains the normalization of the phenotype under conditions of aged cultures, of low humidity, of crowding and of low temperature. The loss of mutant phenotypic expression in each of these cases would be a result of decreased protein synthesis or increased protein utilization during development under suboptimal conditions. Stress induction by environmental change is supported by observations of Sang, McDonald & Gordon (1949), who reported qualitative and quantitative changes in the yeast populations of culture bottles as these cultures aged, and those of Sang (1949*a, b, c*), who reported that the lengths of larval and pupal periods are increased and the weight of emerging adults is reduced when cultures are systematically aged.

The role of the major sex-linked gene in this system is still completely unknown. It may be postulated that this major mutant gene functions in the histoblast in response to physiological changes controlled by the residual genotype. This hypothesis – changes in metabolism under the control of a modifier system which are ultimately responded to by a specific cell under the influence of a specific mutant gene – is similar to that of Bezem & Sobels (1953) for gene control of the *Asy* phenotype. In the developing A^{53g} organism, changes in the metabolic environment of differentiating cells are under the control of the integrated genotype. The genotype of the differentiating cell in turn responds to this environment to form either a normal or abnormal morphological structure.

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