THE PHENOGENETICS OF A SUPER-SUPPRESSOR IN *DROSOPHILA MELANOGASTER*

I. PHENOTYPIC CHARACTERIZATION AND SUPPRESSOR EFFICIENCY

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[*Manuscript received November* 28, 1969]

Summary

An investigation of the phenotypic characteristics of the super-suppressor *su(Hw)*^{2} of *D. melanogaster* was carried out using the suppressible mutant *sc*^{1} together with the scutellar, dorsocentral, and vertical bristle systems. The effect of the suppressor was studied in a series of selection lines differing in both their *sc*^{1} and *sc*$^+$ means.

The suppressor was found to be highly efficient and sensitive to genetic background but insensitive to temperature, developmental rate, and heterochromatin. It would appear that *su(Hw)*^{2} behaves as a complete recessive in all cases but this was only established definitely for the quantifiable mutant *sc*^{1}. The absence of the female-specific characteristics of *su(Hw)*^{2} in males was found not to be due to the presence of the *Y*-chromosome. There was a great similarity between suppressed mutant and wild-type phenotypes which was particularly apparent in the bristle class distributions.

The data are not seen as compatible with the idea that the *su(Hw)*^{2} locus is normally a structural gene for *t*-RNA although the possibility that the translational machinery is in some way involved is very strong.

I. INTRODUCTION

While some 30 suppressor genes are known in *Drosophila melanogaster*, critical studies of their mode of action have not been made and very little use has been made of these suppressors as genetic tools. The most exciting advances with suppressor genes have been made in microorganisms with super-suppressors (Gorini and Beckwith 1966). Two super-suppressors are known in *Drosophila* and of these *suppressor of Hairywing*, *su(Hw)*, is the most intriguing. Discovered, described, and mapped by C. B. Bridges in 1923 this mutant was lost but a second allele *su(Hw)*^{2} was found by E. B. Lewis in 1948 (Lindsley and Grell 1968). Female sterile, locus non-specific, and allele specific, *su(Hw)*^{2} has been shown to suppress at least one allele at each of the following loci: *Bx*, *bx*, *bxd*, *ci*, *ct*, *dm*, *f*, *lz*, *sc*, and *y*. The female sterility has been established as autonomous and is associated with abnormal chromosome behaviour of the nurse cells prior to vitellogenesis (Klug, Bodenstein, and King 1968). These same authors also observed that in the homozygous state *su(Hw)*^{2} delays eclosion by 1 day and behaves as an effective lethal approximately 40% of the time. Other female-specific effects such as a squat body shape and slightly spread wings are also characteristic (Lindsley and Grell 1968).

If the mode of action of *su(Hw)*^{2} can be established it will represent a great step forward in the genetics of *D. melanogaster*. For example, it may allow the molecular classification of known mutants; also the applications to development are unlimited

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(for details see Lee 1969). The aim then of this series of papers is to investigate phenotypic characteristics and interactions of *su(Hw)*^{2} with a view to constructing a hypothesis as to mode of action. This particular series of experiments seeks to quantify the degree of recessiveness of *su(Hw)*^{2}. From this it will be possible to decide if the wild-type allele of the suppressor mediates a competitive reaction. The system chosen involves the *scute* locus together with the scutellar, dorsocentral, and vertical bristles. This system has been extensively quantified (Fraser 1963, 1966; Fraser *et al.* 1965; Rendel 1959; Rendel and Sheldon 1960) and it has even been suggested (Falk 1963) that *scute* and *Hairywing* are two of at least four loci involved in a functional relationship analagous to the operon model proposed by Jacob and Monod (1961). The canalized nature of the *scute* system permits a comparison of the degree of canalization achieved in suppressed *sc*^{1} as opposed to wild type.

The quantitative variation that is associated with a character such as bristle number appears to depend on the presence of wild-type alleles. Substitution of major mutant alleles has the effect of changing this spectrum of quantitative variation (Haskell 1943; Cocks 1954) suggesting that polygenic modifiers are primarily post-translational, since genes affecting transcription or translation would be expected to affect both mutants and their wild-type alleles similarly (Lee and Fraser 1969). Fraser, Erway, and Brenton (1968) have proposed that *sc*^{1} acts as a switch gene activating a wholly new constellation of polygenes, while Rendel (1959) claims that while the polygenes affecting *sc*^{1} are active in the presence of *sc*$^+$ the magnitude of their effects is considerably reduced by the canalized nature of the system, due to the presence of *sc*$^+$ and independent of mean bristle number. In their *sc*$^+$ selection lines Fraser, Erway, and Brenton (1968) have isolated an extra-bristle component (*extra-vert*) which behaves as an allele of *polychaetoid* (Lee, unpublished data). Termed *polychaetoid-verte* (*pyd*^{v}) this factor is completely hypostatic to *sc*^{1} providing an excellent system of testing the efficiency of a suppressor of *sc*^{1} such as *su(Hw)*^{2} and of investigating what polygenic systems are operative in this suppressed *sc*^{1} genotype.

The mutant symbols used in the text are given in the following tabulation:

Mutant	Character Affected	Symbol	Map Position
Achaete	Bristles	*ac*	1– 0·0
Beadex	Wings	*Bx*	1–59·4
Bobbed	Bristles	*bb*	1–66·0
Bithorax	Homeotic mutant	*bx*	3–58·8
Bithoraxoid	Homeotic mutant	*bxd*	3–58·8
Cubitus interruptus	Wings	*ci*	4– 0++
Cut	Wings	*ct*	1–20·0
Deficiency of yellow, achaete, scute		*Df(1)260–1*	1– 0·0
Diminutive	Body size	*dm*	1– 4·6
Forked	Bristles	*f*	1–56·7
Lozenge	Eye shape	*lz*	1–27·7
Polychaetoid-verte	Bristle number	*pyd*^{v}	3–39·0
Scute	Bristle number	*sc*	1– 0·0
Scute deficiency	Bristle number	*Df(1)sc*$^{10-1}$	1– 0·0
Silver	Coloration	*svr*	1– 0·0
Suppressor of Hairywing		*suHw*	3–54·8
Translocation (2; 3) apterous-Xasta	Wing shape	*T*(2;3) *ap*^{xa}	
Attached *X* chromosomes		$\overline{XX}$	
Yellow	Body colour	*y*	1– 0·0

II. Materials and Methods

The mutant $su(Hw)^2$ was backcrossed into the following sc^1 stocks:

Designation	Reference	Description
A	Miller and Fraser (1968)	A line selected for extra scutellar bristles prior to substitution of sc^1; homozygous pyd^v. Formerly denoted 1,1
B		Replicates of 1,1 with different bristle means
C		Formerly denoted 1,9 and 1,18
D		A replicate of 1,1 which may not be homozygous pyd^v. Formerly denoted 1,4
E		A replicate of 1,1 not containing pyd^v. Formerly denoted 1,6
F	Lee and Fraser (1969)	A line formed by crossing lines B and C, then selecting for reduced sex dimorphism of scutellar bristles. Formerly denoted R1
G		A line constituted at the same time and in the same fashion as line F but unselected. Formerly denoted Con
Re^1	Rendel (1959)	A line selected for increased scutellar bristle number in the presence of sc^1
Re^{11}	Rendel, Sheldon, and Finlay, unpublished data	From a different base stock but selected in the same fashion as Re^1
Re^{111}		A line selected for decreased scutellar bristle number in the prescence of sc^1

The method of backcrossing was as follows:

$$Xa/su(Hw)\ bxd \times y^2sc^1;\ +/+ \quad \text{Stock males}$$

$$\downarrow$$

$$y^2sc^1;\ +/suHw\ bxd \times y^2sc^1/y^2sc^1 \quad \text{Stock females}$$

$$\downarrow$$

Mate progeny *inter se* and select $suHw/suHw$ males on suppression of y^2

This scheme of backcrossing for two generations, mating *inter se*, selecting homozygous males and backcrossing for two more generations was carried out for 19 effective generations of backcrossing with lines F, G, and B, for 12 backcross generations with line A, and for 9 backcross generations with lines C, D, and E. 100 females and 100 males homozygous for $su(Hw)^2$ were scored for scutellar bristle number every generation starting at generation eight. The dorsocentral and vertical bristle systems were scored following the final backcross generation in lines F, A, and C. In all of these lines sc^1 is marked by y^2 which was indispensable as an independent indicator of $su(Hw)^2$ since it is also suppressible. The three lines obtained from Rendel contain no independent suppressible gene and were only carried for six backcross generations.

The original source of $su(Hw)^2$, a *bx bxd* $su(Hw)^2$ chromosome obtained from Pasadena, proved virtually lethal when homoyygous, greatly facilitating recovery of crossovers. From the third backcross generation onwards no bithorax phenotype was seen while individuals homozygous for both third chromosome mutants $su(Hw)^2$ (54·8) and pyd^v (39·0) were common from the fifth backcross generation onwards.

A test for a heterozygous effect of $su(Hw)^2$ was carried out following backcross generation eight in line F. The investigation was carried out at two densities: (1) single pairs in quarter-pint cream jars and (2) 50 pairs in quarter-pint cream jars.

Two qualitative tests were also carried out to test the efficiency of suppression by $su(Hw)^2$. The first involved females heterozygous for sc^1 and a deficiency. The two deficiencies used were *Df* (1) sc^{10-1}, deficient for the single band 1B2, and *Df*(1)260–1, a terminal deficiency of *y*, *ac*, and *sc* but not *svr*. Both heterozygotes are completely devoid of scutellar bristles and it was of interest to know if a single dose of sc^1 was sufficient to respond to the suppressor. Secondly a multiple-tester stock carrying the suppressible mutants y^2, sc^1, ct^6, and f^1 was synthesized with the purpose of investigating whether the efficiency of $su(Hw)^2$ is in any way affected by the amount of "work" it is called upon to do. While all of these experiments were carried out at room temperature (25°C) the temperature sensitivity of $su(Hw)^2$ was investigated with cultures raised at both 18 and 30°C.

Kuwano, Ishizawa, and Endo (1968) have reported restriction of the activity of super-suppressors in *Escherichia coli* due to mutation to streptomycin resistance, a phenomenon interpreted as due to alteration of ribosome structure (Otsuji and Aono 1968). Since the *bobbed* region and the *Y*-chromosome in *D. melanogaster* have been implicated in the production of ribosomal RNA (Ritossa, Atwood, Spiegelman 1966) it was decided to measure the efficiency of $su(Hw)^2$ both in the presence of *bobbed* mutants and in the absence of the *Y*-chromosome. Females from an attached *X*, attached *X*–*Y* stock which contained no free *Y*-chromosomes were used to produce males lacking a *Y*-chromosome in the following manner:

$$\underset{\text{females}}{Xa/su(Hw)^2;\ XX} \times \underset{\text{males}}{Xa/su(Hw)^2;\ y^2sc^1}$$

$$\downarrow$$

$$XO \text{ males } su(Hw)^2/su(Hw)^2;\ y^2sc^1$$

In all experiments flies were grown in quarter-pint milk bottles containing *c.* 30 ml standard cornmeal–molasses–yeast–agar medium. All backcross generations were initiated with 20 males and 30 virgin females in each of two replicates which were pooled prior to scoring or selecting individuals for the next generation.

III. Results

(i) *The Backcross Lines*

The mutant $su(Hw)^2$ was successfully backcrossed into all seven lines carrying the independent marker y^2 for at least nine generations. That this was sufficient to restore the background genotype is demonstrated in Figure 1 in which are depicted the

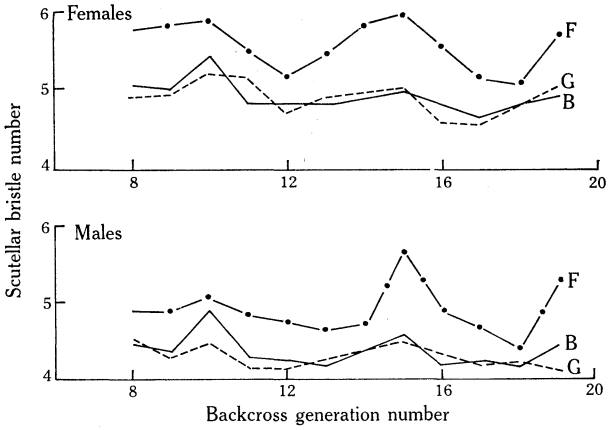

Fig. 1.—Mean scutellar bristle number in successive backcross generations.

results of 18 backcross generations with lines F, B, and G. From the ninth to the eighteenth generation no directional change in bristle mean was observed despite

considerable fluctuation. This fluctuation is thought to be due to the extreme sensitivity of the pyd^v mutant to variations in temperature and density (Fraser, Erway, and Brenton 1968). Table 1 presents for these seven lines the three levels of scutellar bristle expression, each of which represents a distinct genetic system namely, sc^+, sc^1, and suppressed sc^1. The efficiency of $su(Hw)^2$ can be seen to vary from 70 to

TABLE 1

EFFECT OF $su(Hw)^2$ ON SCUTELLAR BRISTLE NUMBER IN FEMALES AND MALES OF THE SEVEN sc^1 SUBSTITUTED SELECTION LINES

Genetic System	Substituted Selection Line						
	F	G	B	C	A	E	D
				Females			
sc^+ (Oregon-R)	6·36	6·59	7·28	6·43	7·18	4·81	5·05
Suppressed sc^1	5·51	4·84	4·94	5·88	6·51	4·42	5·17
sc^1	2·15	1·73	1·66	1·56	2·12	2·36	2·42
Efficiency of suppression (%)	80·9	73·5	67·9	91·4	90·8	91·8	102·3
				Males			
sc^+ (Oregon-R)	5·54	5·86	6·31	5·88	6·02	4·26	4·71
Suppressed sc^1	4·66	4·22	4·28	5·22	5·84	4·16	4·23
sc^1	1·86	0·85	0·82	0·55	1·89	2·19	2·16
Efficiency of suppression (%)	84·3	72·1	67·9	88·8	92·8	97·6	89·8

100% with close agreement of male and female data. Line D appears somewhat anomalous and this is attributed to the fact that it is the only line thought to be segregating for a major bristle number component (pyd^v) whose frequency could have altered due to random drift during backcrossing. The efficiency of suppression characteristic of any one line is not predicted by either the sc^+ mean or the sc^1 mean of the line. This is illustrated strikingly by Figure 2 in which the ranking of the array of lines differs for each of three genetic systems. Not only are the relative means not conserved but neither are the patterns of sex dimorphism. The vertical and dorsocentral bristles, as seen in Table 2, follow the same pattern as the scutellar bristles, confirming the impression that wild-type conditions with respect to the *scute* locus are being approximated in the presence of the suppressor.

The canalization-induced inertia that characterizes scutellar bristle distributions is still very much apparent as shown by Figure 3. There is still an accumulation of individuals in the four- and six-bristle zones, indicating the presence of strong canalization forces. Thus canalization is a phenotypic phenomenon which does not depend upon the presence of the sc^+ gene.

Despite the different origin of Rendel's lines, a similar response in suppressed sc^1 flies was evident after six backcross generations:

Line	Mean Scutellar Bristle Number	
	Females	Males
Re^1	4·47	4·32
Re^{11}	4·42	4·09
Re^{111}	4·01	4·00

Lines Re^{1} and Re^{11} began to show extra bristle phenotypes in response to *su(Hw)*^{2} as early as the third backcross generation while line Re^{111}, which occasionally has missing bristles in the presence of *sc*$^{+}$, did not exhibit such a phenotype in the presence of

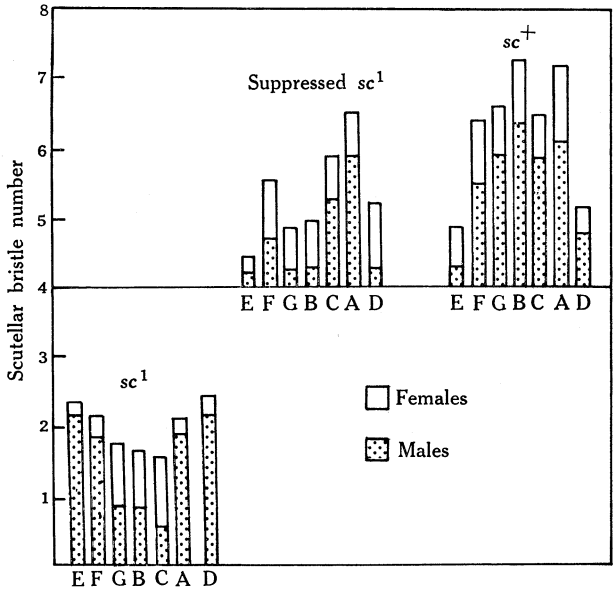

Fig. 2.—Selection line histograms of scutellar bristle number at the three genetic levels.

su(Hw)^{2} (*sc*^{1}) up to the sixth backcross generation. The absence of an independent indicator for *su(Hw)*^{2} (such as the suppressible mutant *y*^{2}) rendered the Re lines

TABLE 2

COMPARISON OF THE DORSOCENTRAL AND VERTICAL BRISTLE SYSTEMS AT TWO LEVELS

Line	Sex	Suppressed *sc*^{1}		*sc*$^{+}$	
		Dorso-centrals	Verticals	Dorso-centrals	Verticals
A	Female	4·52	6·26	5·08	7·10
F	Female	4·28	6·10	4·92	6·84
A	Male	4·12	6·13	4·92	6·85
F	Male	4·37	6·27	4·81	6·72

extremely difficult to work with. Accordingly they were discontinued after six backcross generations. Occasionally extra dorsocentral and vertical bristles were seen in Re^{1} but these bristle types were not scored systematically.

(ii) *Efficiency of Suppression*

The scutellar bristle mean of Re^{111} (sc^1) is well below 1 for females and approaches zero for males due to the presence of modifiers, yet there is still more bristle-forming potential present than in sc^1/deficiency females. Such females heterozygous for sc^1 and either *Df(1)260-1* or *Df(1)*sc^{10-1} have no scutellar bristles and, under conditions of high temperature, *Df(1)260–1* is occasionally dominant to sc^+, resulting in missing bristles. When either deficiency is heterozygous with the non-suppressible allele sc^5, *su(Hw)*^{2} has no effect on the bristle phenotype and therefore no effect on the deficiency, but *su(Hw)*^{2}, when homozygous, restores all scutellar bristles to both sc^1/deficiency heterozygotes. This high level of efficiency is in general agreement with that found for the selection lines and also demonstrates that a single dose of a suppressible mutant is sufficient to permit full suppression in the diploid condition.

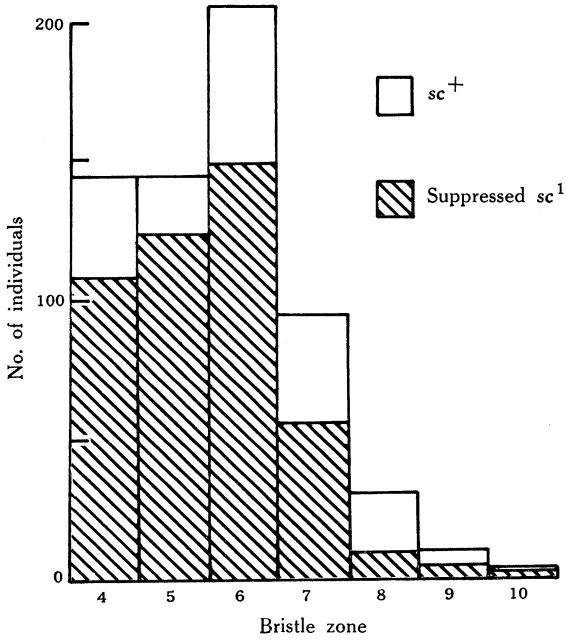

Fig. 3.—Comparison of sample sc^+ and suppressed sc^1 distributions illustrating the conservation of canalization.

In the multiple-tester stock y^2 sc^1 ct^6 f^1, which is homozygous for *su(Hw)*^{2}, the same degree of suppression for all four mutants as in the single state was observed suggesting that the efficiency of suppression is more probably determined by the ability of the mutant concerned to respond to the "suppressor substance" than by the amount of "suppressor substance" available. In other words, the absence of any saturation effect would predict that *su(Hw)*^{2} could simultaneously suppress any number of mutant alleles.

(iii) *Factors affecting Suppression*

(1) *Heterozygosity.*—In the following tabulation the mean scutellar bristle number for 1000 male and 1000 female flies of line F homozygous and heterozygous for

the wild-type allele of $su(Hw)^2$ and reared at high and low densities are compared:

	Females	Males
Reared at low density		
$su(Hw)^2/+$	2·10	1·83
$+/+$	2·20	1·97
Reared at high density		
$su(Hw)^2/+$	2·21	1·87
$+/+$	2·15	1·96

There appears to be no consistent difference attributable to a single dose of $su(Hw)^2$ and because of the extreme sensitivity of bristle number in sc^1 flies. It can be concluded that $su(Hw)^2$ is a complete recessive with respect to sc^1. Although the suppressible alleles y^2, ct^6, f^1, and *bxd* were not examined quantitatively with heterozygous $su(Hw)^2$, observations on some 500 such heterozygotes detected no effect.

(2) *Temperature.*—Crosses at 18 and 30°C were set up such that all non-*apterous*-*Xasta* flies would be homozygous for $su(Hw)^2$. All such flies examined at both temperatures were non-*y* and non-*sc*, indicating that the suppressor is fully operative at both temperatures. At 30°C mortality of flies is normally quite high but flies homozygous for the suppressor seem to be particularly sensitive suffering up to 95% mortality. Scutellar bristle means for sc^1 flies homozygous for $su(Hw)^2$ were little influenced by temperature as seen in the following tabulation ($n = 2000$):

	Rearing Temperature		
	18°C	25°C	30°C
Females	4·06	4·01	4·01
Males	4·02	4·00	4·00

The extreme sensitivity of sc^1 to temperature (Child 1935*a*, 1935*b*) was no longer apparent among suppressed sc^1 flies.

(3) *Heterochromatin.*—The absence of a *Y*-chromosome in male *D. melanogaster* lengthens the time of development by approximately 1 day and increases the scutellar bristle number of sc^1 flies considerably (Mampell 1965). Neither of these two characteristics appeared to influence the suppression of sc^1 by $su(Hw)^2$ in *XO* males. A *bobbed* mutant (bb^{68F}) arose spontaneously in one line and was isolated. The features of this mutant were the extreme abnormal effect on the abdomen of females and the slight effect on bristle size. Once again neither the presence of this mutant nor of bb^N (obtained from Pasadena) affected the suppression of $y^2\,sc^1$ by $su(Hw)^2$. However, the female-specific characters of squat body shape and spread wings induced by homozygous $su(Hw)^2$ were not seen in *XO* males.

IV. Discussion

The experimental results indicate that $su(Hw)^2$ is highly efficient, sensitive to genetic background, but insensitive to temperature, developmental rate, and heterochromatin and is completely recessive, at least with respect to sc^1. Its efficiency was demonstrated not to be affected by increasing the number of suppressible alleles present in one genome or by replacing one of a pair of suppressible alleles with a deficiency. Suppressed mutants have been shown to lose typical characteristics such as the temperature sensitivity and poor canalization of sc^1. Finally it was shown that lack of the *Y*-chromosome is not responsible for the female-specific effects of $su(Hw)^2$.

A vital question concerning the influence of genetic background is the identification of the modifiers concerned. Certainly pyd^v and at least some of the extra bristle polygenes manifest in sc^+ are being expressed in suppressed sc^1 and since we know that the polygenes affecting sc^1 are largely distinct from those affecting sc^+ (Haskell 1943; Cocks 1954; Lee and Fraser 1969), it would appear from the suppressed sc^1 array (Fig. 1) that yet other specific modifiers of $su(Hw)^2$ are operative. It is unlikely that these affect the quantity of suppressor substance or the ability of suppressible alleles to respond to it since evidence against these factors being limited was found. It is difficult to conceive an experiment in which selection for suppressor efficiency could be practised without selection of modifiers affecting the character under study but such an experiment would represent a positive approach to isolating specific modifiers of $su(Hw)^2$.

The conservation of sc^+-type canalization and the demonstration of canalization as a phenotypic or developmental process or both as opposed to a genotypic one is not surprising but does serve to underline the basic similarity between sc^+ and suppressed sc^1.

The high lethality of homozygous $su(Hw)^2$ reported by Klug, Bodenstein, and King (1968) was not always in evidence during the experiments reported in the previous section and it is proposed:

(1) That as a female-sterile mutant, natural selection will favour close linkages of $su(Hw)^2$ with lethals and semi-lethal genes.
(2) Genetic background may determine the lethality of $su(Hw)^2$ as in synthetic lethal systems.

The operation of such synthetic lethal systems in the backcross lines may be responsible for the observed variations in efficiency.

The expression of almost all mutant genes in *D. melanogaster* is sensitive to changes in temperature, media conditions, and developmental rate (Plunkett 1932). Such an observation could be due to the types of mutants generally studied. Mutants which produce an inefficient enzyme or gene product or which disturb the timing of developmental processes would be expected to behave in this fashion. Such mutants are also generally hypomorphic, i.e. in the absence of the wild-type allele their mutant effect is diminished with increasing gene dosage (Muller 1950). Clearly $su(Hw)^2$ does not seem to belong to this category. Another feature of most *D. melanogaster* mutants is that they are seldom clearly recessive. Mutant expression in the heterozygous condition can be induced by temperature shock (Landauer 1958), selection (Goldschmidt 1935), position effect (Gardener 1942), the presence in heterozygous or homozygous condition of other mutants with similar effects (Green and Oliver 1940; Neel 1941), by substitution in high selection lines (Miller and Fraser 1968), or by close examination in other sensitive genetic backgrounds. The finding, therefore, that $su(Hw)^2$ is a complete recessive serves once again to isolate it from the majority of *D. melanogaster* mutants. Clearly then the gene product of the wild-type allele is completely effective in competing against the gene product of $su(Hw)^2$ (or suppressor substance) and this argues strongly against the locus being responsible for *t*-RNA production.

The suppressor genes in microorganisms that have been found to produce *t*-RNA's with altered anti-codons are almost all dominant. Even if these altered *t*-RNA's have to compete with other *t*-RNA's, the small number of "sense" molecules

they produce should be sufficient to offset the mutant phenotype. There is no reason to believe that the situation would be otherwise in *D. melanogaster*. Eggertsson and Adelburg (1965) have suggested a possible mechanism for a recessive suppressor. If the suppressor locus normally controls the production of an enzyme which modifies *t*-RNA structure (e.g. by methylation) or affects amino acid coupling (synthetase) the absence of this enzyme may result in coding mistakes of the type leading to multiple suppression.

With respect to the morphological characteristics of *su(Hw)*^{2} the simple hypothesis that it produces a substance which corrects mutant genes and at the same time interferes with normal genes is tenuous. It is unusual that such phenotypes are restricted to females particularly since it was shown that the *Y*-chromosome is not involved. Restriction of sterility to females as in *su(Hw)*^{2} is quite a common occurrence and this could be a clue to the manifestation of the spread wings and squat body shape only in females. However, *su(Hw)*^{2} has been discovered on two independent occasions and in both instances has had the same pleiotropic effects. This is indeed strong evidence that a common substance is involved. Furthermore sex-limited mutants are not uncommon in *D. melanogaster* and mutants do exist which have the same pleiotropic effects as *su(Hw)*^{2}. Also, with respect to genetic disturbance female fertility is one of the most sensitive characters in *D. melanogaster*.

Suppressor of *vermilion*, the only other super-suppressor known in *D. melanogaster*, suppresses solely pigment mutants, viz: *vermilion*, *sable*, and *purple*. A number of alleles are known and Glass (1957), among others, has presented a reasonable explanation of its mode of action in terms of intermediary metabolism. This leaves *su(Hw)*^{2} as the only candidate for homology with the translational suppressors found in microorganisms.

Now that some of the major phenotypic responses of *su(Hw)*^{2} have been established and its behaviour in a number of circumstances predicted it is hoped that it will be possible to apply more subtle tests which will perhaps provide further insight into the mode of action of this most promising mutant.

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