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

III.* SUPPRESSION AT INDIVIDUAL LOCI

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[Manuscript received 10 August 1972]

Abstract

Seventy-two previously untested mutants of *D. melanogaster* were tested for suppression by *su(Hw)*^{2}. Of these, only six were found to be suppressible while the suppressibility of one previously tested mutant, namely *Bar*, is called into doubt. It is suggested that there are functional differences between the three types of loci classified by their response to *su(Hw)*^{2}.

I. INTRODUCTION

The first paper in this series (Lee 1970) presented a phenotypic characterization of the super-suppressor *su(Hw)*^{2}, while the second investigated the interrelationship between suppression and back-mutation in *Drosophila melanogaster* (Lee 1972*b*). From this data it was postulated that *su(Hw)*^{2} is a codon-specific suppressor operating at the level of information transfer and despite a number of peculiarities this mutant is thought to be analogous to the super-suppressors of *E. coli* (Gorini and Beckwith 1966).

The next question to be approached concerned the scope or range of activity of the suppressor. Not only are the widespread effects of super-suppressors in microorganisms not expected in higher organisms due to increased complexity and compartmentalization, but such restrictions to time or tissue specificity as can be located should shed further light on the role of the wild-type allele of *su(Hw)*^{2}. In order to further expand the spectrum of suppression a large number of alleles were tested with particular attention being paid to loci at which suppressible alleles are known to occur. Of the 213 alleles so far tested (Lindsley and Grell 1968) 14 have been shown to be suppressible. These are distributed over 12 loci. A thorough examination of the *white* locus was also undertaken because of the large number of distinguishable point mutations that exist and because much is known of the fine structure of this region.

II. MATERIALS AND METHODS

The suppressor *su(Hw)*^{2} is a third-chromosome mutant and it was combined with all but third-chromosome mutants by means of the balancer technique (Lee 1972*b*). In most instances

* Part II, *Aust. J. biol. Sci.*, 1973, **26**, 189–99.

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M5 (*Basc*) was used to balance the first chromosome, and *Xasta* the second and third chromosomes with occasional use being made of *TM1*. All third-chromosome mutants tested for suppression were marked by a closely linked, easily detected mutant and, to avoid complications caused by double crossovers, all progeny were examined and only if marked "non-mutant" flies outnumbered other crossover classes was it concluded that suppression was occurring. The use of an indicator phenotype, usually the suppressible y^2 mutant, was supplemented by the use of *bx* and *stb* as markers of *su(Hw)*^{2}. Viability problems were encountered with some of the balancers, particularly *FM1* and some marker–mutant combinations were not practical, such as *Ubx* with *bx* or *bxd* and *Stb* with bristle mutants, but satisfactory replacements were found. All crosses were executed in duplicate and at least 200 progeny of 10 pairs of parents per quarter-pint bottle were examined and all phenotypes present scored.

III. Results

The results of the suppression tests are summarized in Table 1. Seventy-two previously untested mutants were examined with only six proving suppressible, three at the *cut* locus and three at the *scute* locus.

TABLE 1

SUMMARY OF SUPPRESSION TESTS

Mutant	Previously tested alleles (Lindsley and Grell 1968)		New alleles tested in this report	
	Suppressible	Non-suppressible	Suppressible	Non-suppressible
scute	3	3	3	2
scute rearrangements	—	5	—	2
yellow	1	4	—	11
forked	1	1	—	3
Hairy wing	1	—	—	3
cut	1	1	3	—
lozenge	1	8	—	1
white	—	14	—	6
Bar	1	—	—	5
Lethals	—	—	—	17
Other alleles	6	163	—	17

The *su(Hw)*^{2} suppressibility of one previously tested mutant is called into doubt while the description of another, as only partially suppressed, is shown to be inaccurate except in certain stocks containing a third mutant. In addition all other alleles previously reported as suppressible were re-tested and previous results confirmed. The one exception was *lozenge*^{1} which inadvertently was not re-tested after the first test failed.

(*a*) *The* scute *Locus*

The five point mutations previously untested for suppression were examined with the result that sc^{3B}, sc^{L3}, and sc^{L6} were found to be suppressible but negative results were obtained with sc^{28} and sc^{67B5}. The response of these five alleles and that of four other *scute* mutants to the extra-bristle mutant pyd^v are presented in Table 2 where it can be seen that the epistasis of *scute* to pyd^v, which is directly

proportional to the severity of the *scute* allele, is retained to some extent in the presence of *su(Hw)*^{2}.

TABLE 2

RESPONSE OF *scute* ALLELES TO *su(Hw)*^{2} AND *pyd*^{v}

scute allele	Description	Expression of *pyd*^{v} alone*	Expression of *pyd*^{v} *with* *su(Hw)*^{2} (%)
sc +	Wild type	Complete	100
sc^{D1}	Weak allele	Substantial	70–90
sc^{D2}	Weak allele	Substantial	70–90
sc^5	Weak allele	Good	Unchanged
sc^1	Moderate allele	Moderate	70–90
sc^{L6}	Moderate allele	Low	60–80
sc^{3B}	Strong allele	Slight	50–70
sc^{L3}	Strong allele	Very slight	10–30
sc^{28}	Strong allele	No observable	Unchanged
sc^{67B5}	Strong allele	No observable	Unchanged

* Substantial expression = regular twinning of scutellar, dorsocentral and vertical bristles;

Good expression = regular twinning of dorsocentral and vertical bristles;

Moderate expression = regular twinning of vertical bristles;

Low expression = occasional twinning of dorsocentral and vertical bristles;

Slight expression = rare twinning of vertical bristles.

The alleles designated *1*, *D1*, *D2*, and *L6* are not differentiated by this method. Although all four are spontaneous in origin (Lindsley and Grell 1968) *sc*^{D2} arose in a yellow stock and *sc*^{D1}, which is associated with a *Hairywing* effect, arose simultaneously with a yellow mutant. Neither of these alleles had previously been separated from these associated phenotypes but when suppressed by *su(Hw)*^{2} all three phenotypes persist, indicating that, although closely linked to *scute*, they are not pleiotropic effects of their respective *scute* alleles. There is no current reason therefore to regard these two alleles as other than re-occurrences of the original *sc*^{1} mutation. The spoon-wing phenotype associated with *sc*^{L3} was likewise found to persist when the *scute* phenotype was suppressed.

Combinations of the non-suppressible alleles *sc*^{5}, *sc*^{3B}, *sc*^{28}, and *sc*^{67B5} with *sc*^{1} were found to exhibit wild-type phenotypes in response to *su(Hw)*^{2} while combinations of *sc*^{1}, *sc*^{D1}, *sc*^{D2}, and *sc*^{L6} with the deficiency *sc*$^{10-1}$ also gave a full response to the suppressor but the *sc*^{L3}/*sc*$^{10-1}$ combination had a reduced scutellar bristle mean of 3·81. The small *sc*$^{10-1}$ deficiency and the large *sc*$^{260-1}$ deficiency are both unaffected by *su(Hw)*^{2}. *Scutoid*, a dominant second chromosome mimic of *scute* (Lee and Fraser 1969), also tested negatively.

(*b*) *The* yellow *Locus*

The following alleles were all tested with no response to *su(Hw)*^{2}: *y*^{bl}, *y*^{59b}, *y*^{31d}, *y*^{51g}, *y*^{4}, *y*^{50k22}, *y*^{td}, *y*^{16}, *y*^{329}, and *y*^{3P}.

The literature (e.g. Lindsley and Grell 1968) has always reported the suppression of y^2 by *su(Hw)* as partial with only the yellow wing colour being suppressed, a finding not supported by this current study in which almost complete (i.e. overlapping wild-type) suppression of the yellow body colour over a large range of stocks was found. Two stocks, however, showed unexpectedly poor suppression of y^2. These were a y^2 *cv ct* lz^{50} *e v g odsy* stock and a y^2 *ec cv v f* stock. The only two mutant genes common to both stocks are *vermillion* and *crossveinless* and since *vermillion* does not show this effect in other stocks a y^2 *cv* $ct^6 f^1$ stock was examined and the same poor suppression of y^2 noted. This suppression interference of *cv* appears restricted to y^2 because ct^6 and f^1 continue to show normal suppression.

(*c*) *The* Hairy wing *Locus*

Hw^{49c} and Hw^{59G} (Lee 1972*a*), which are both point mutations, failed to respond to $su(Hw)^2$ as did the *Hairy wing* phenotypes of sc^{D1}, sc^8, and sc^{10-1}. Morgan *et al.* (1941) gave the name *Su(1) Hw* to an inversion which separates the two duplicated bands in the original *Hairy wing* mutation. However, this inversion does not behave as a suppressor but rather a partial reversion and has since been renamed Hw^2. This mutant overlaps wild-type to such a degree that no definite statement about its response to $su(Hw)^2$ can be made although full suppression of 100 Hw/Hw^2 females examined was recorded.

(*d*) *The* cut *locus*

A new allele designated ct^{67s} was found (Lee 1972*a*) and when tested proved to be suppressible by $su(Hw)^2$. This new allele was phenotypically similar to ct^1, which also proved suppressible when tested. The alleles ct^1 and ct^{67s} are distinguished from ct^6, the original suppressible allele, by their concomitant effects on the eye, abdomen, and antenna, all of which are suppressed. Flies carrying ct^6 together with its enhancer $divers^2$ may occasionally show slight nicks in the wings in the presence of homozygous $su(Hw)^2$, indicative of incomplete suppression. A similar partial suppression is seen with ct^K, an allele associated with fine *minute*-like bristles. Both the *cut* wing phenotype and the bristle phenotype are suppressed by homozygous $su(Hw)^2$ while incomplete suppression of the wing phenotype alone is seen with heterozygous $su(Hw)^2$. This is the first example of a heterozygous effect of $su(Hw)^2$ and suggests that the *ct* locus contains the most sensitive of all alleles so far studied.

(*e*) *The* Bar *locus*

The reports of Bridges (1932) for *su(Hw)* and Lewis (Lindsley and Grell 1968) for $su(Hw)^2$ concerning the suppression of *Bar* could not be confirmed. Examination of 2000 *B/.* males homozygous $su(Hw)^2$ together with an equal number of *B/.* males heterozygous $su(Hw)^2$ from the same cultures revealed no difference in eye size. In this case the *Bar* allele used was that carried on the Muller 5 chromosome. An exhaustive study of all alleles and combinations of *Bar* was made in males and heterozygous and homozygous females. The suppressible allele *forked* was used as an indicator of $su(Hw)^2$ homozygosity and eye-size comparisons were made between *forked* and non-*forked* individuals from the same culture. Since *forked* is a closely linked (0·3 map units)

marker of *Bar* the existence of non-*forked Bar* flies is indicative of lack of suppression of *Bar*. The results were as follows:

B/., *B*/+, *B*/*B*	No effect
B^i/., B^i/+, B^i/B^i	No effect on size or roughness; some shape modification
BB/., *BB*/+, *BB*/*BB*	No effect
B^3/., B^3/+, B^3/B^3	No effect
BB^{36b}/., BB^{36b}/+, BB^{36b}/BB^{36b}	No effect on eye size but a slight difference in shape noted in heterozygous females
B^iB^i/., B^iB^i/+, B^iB^i/B^iB^i	A slight difference in shape noted in some males and heterozygous females

Scoring for size was done as follows. One *forked* male and one non-*forked* male were chosen at random and compared under the binocular microscope. If one fly had a larger eye size than the other then it was placed in a separate container. If no difference was noted the pair were discarded. When 100 flies were in the container these were then scored for the character *forked* bristles and deviations from a 1 : 1 ratio were subject to a χ^2 test. All tests were non-significant at the 1% level.

These results, covering as they do a range of eye sizes, support the initial observation that *su(Hw)*^{2} is without effect on *Bar* and so contradict all earlier reports that *Bar* is suppressible. As previously noted (Lee 1970), *su(Hw)*^{2} has a slight effect on eye shape producing a slightly smaller, rounded eye most noticeable in females, and considerably exaggerated in the presence of the mutant *supact* (Lee 1972*b*). Alleles of *Bar*, on the other hand, result in a pronounced oblong-shaped eye and any rounding effect superimposed upon this could mistakenly be interpreted as suppression. This effect was only seen in some females of the B^i, B^iB^i, and BB^{36b} stocks and in no instance was the magnitude of the effect greater than 10%.

(*f*) *The* white *Locus*

The remaining untested *white* alleles, namely *ivory*, *coloured*, *garnetoid*, *crimson*, *spotted*, and the *white-apricot* reversal of Mossig, all failed to respond to *su(Hw)*^{2}.

(*g*) *Alleles at Other Loci*

The following alleles of various genes were tested and found not to be suppressible: *bobbed-Novitski*, *crossveinless*, *deep orange*, *forked*^{3N}, *forked*^{5}, *forked*$^{257-4}$, *garnet*^{4}, *halfway* (a lethal), *lozenge*^{50}, *maroon-like*, *miniature*, *outstretched-smallwing*, *Xasta*, and *zeste*. Also testing negatively were 16 of Novitski's (1963) 17 non-autonomous, sex-linked lethals [1(1)EN(1→10, 10*a*→16)]. The following mutants arising during the course of these experiments also tested negatively: *bobbed*^{68F}, *kidney*^{66}, *apterous*^{68E}, and recessive alleles of *Serrate* and *Delta*.

IV. Discussion

If *D. melanogaster* is the most complex organism in which informational suppressors have been more than just postulated, then it also has the most cryptic suppression pattern. One reason for this is that unlike the situation in microorganisms, informational suppressors in *D. melanogaster* are not integrated into the genetic system from an evolutionary point of view. Two attributes of microorganisms are responsible for the evolutionary importance of suppressors, namely haploidy and

lack of complexity, neither of which *D. melanogaster* can offer. In the diploid organism, newly arising mutations are sheltered from immediate selection pressure in the heterozygote and so are conserved for possible future use. A suppressor-carrying *E. coli* strain plays a similar role which means that the suppressor can have a selective advantage. The undifferentiated nature of microorganisms permits a super-suppressor to affect alleles at most loci but in more sophisticated organisms, where development is a complex series of tissue-specific and coded sequential operations, the influence of a viable suppressor is necessarily limited.

Superficially loci in *Drosophila* can be divided into three groups:

(1) loci at which suppressible alleles are common, e.g. *sc*, *ct*;

(2) loci at which such alleles are rare, e.g. *y*; and

(3) loci at which they do not occur at all, e.g. *w*.

This division may reflect the nature of the suppressor, the type of viable allele permissible at a locus, or the time at which the loci are translated. Some evidence against this latter possibility is that the absence of dead larvae indicates that the lethal effect of $su(Hw)^2$ is operative early in development (Lee, unpublished data), the suppressible allele y^2 is autonomous throughout development (Stern 1956), while the female sterility of $su(Hw)^2$ is imposed very late in development (Klug *et al.* 1968). Thus the suppressor is active at least in some tissues throughout all stages of development. The possibility of tissue specificity is consistent with the finding of Klug *et al.* (1968) that $su(Hw)^2$ is autonomous in development.

The use of a suppressor allows some division of alleles within a locus, such as *scute*, where the degree of suppression is proportional to the severity of the allele suppressed. An allele such as ct^K, the only allele responding to heterozygous $su(Hw)^2$, is unlikely to be structurally different from alleles such as sc^1 (Lee 1970) where there is not even the slightest response to heterozygous $su(Hw)^2$. This difference could be allele or tissue specific, respectively determined by the sensitivity of the suppressible allele or the differential activity of the suppressor.

The application of a suppressor gene as a genetic tool in the resolution of the suspected pleiotropic effects of mutants was illustrated with sc^{D1}, sc^{D2}, and sc^{L3} which were shown to be separable from their respective associated phenotypes while ct^K was seen to exhibit genuine pleiotropic effects. This method allows exact conclusions to be drawn about whether two effects are due to one mutant or not, while the arduous crossing-over technique can lead only to statistical statements.

It has been suggested why the erroneous description of $su(Hw)^2$ as a suppressor of *Bar* has persisted in the literature but the source of the original report has proved elusive. No mention is made of *Bar* in the original report of C. B. Bridges in the yearbook of the Carnegie Institute of Washington of 1923. Bridges and Brehme (1944) is the first reference source describing *Bar* as a *su(Hw)*-suppressible allele although the only reference supplied is Bridges' (1932) paper which makes no mention of *Bar*. That Bridges had much unpublished data on *su(Hw)* is suggested by a reference in a paper by Schultz and Bridges published in 1932 to a paper by Bridges entitled "Specific suppressors in *Drosophila*", scheduled to appear in the *Quarterly Reviews of Biology* in 1932, but which in fact never appeared. Part of this unpublished work may have included work on *Bar*, or Brehme who earlier worked on

other *Bar* suppressors may have studied *su(Hw)*. A more likely possibility, however, is that the report stems from a misinterpretation of Morgan (1929). Although Morgan does not specifically identify his suppressor as *su(Hw)*, there is no doubt that it is the same mutant since both suppressors are female sterile, both suppress *scute* and *forked* to the same degree, both are autosomal recessives mapping in the same vicinity on chromosome III, and both have a lethal effect ranging from 10 to 30% when homozygous. Morgan studied suppression of *forked* using *Bar* as a marker and refers often to "non-*forked Bar* flies". In fact he examined in excess of 8000 *f B*; +/*su(Hw)* flies segregating in cultures with a similar number of their *f B*; *su(Hw)*/*su(Hw)* sibs and, while discussing the suppression of *forked* at length, makes no mention of any suppression effect on *Bar*.

V. References

BRIDGES, C. B. (1932).—Specific suppressors in *Drosophila*. Proc. 6th Int. Congr. Genet. Vol. 2. pp. 12–14.

BRIDGES, C. B., and BREHME, K. S. (1944).—"The Mutants of *Drosophila melanogaster*." (Publs Carnegie Instn No. 552.)

GORINI, L., and BECKWITH, J. R. (1966).—Suppression. *A. Rev. Microbiol.* **20**, 401–22.

KLUG, W. S., BODENSTEIN, D., and KING, R. C. (1968).—Oogenesis in the suppressor of *Hairywing* mutant of *Drosophila melanogaster*. I. Phenotypic characterisation and transplantation experiments. *J. exp. Zool.* **167**, 151–6.

LEE, G. L. G. (1970).—The phenogenetics of a super-suppressor in *Drosophila melanogaster*. I. Phenotypic characterization and suppressor efficiency. *Aust. J. biol. Sci.* **23**, 645–55.

LEE, G. L. G. (1972*a*).—Note in *Drosophila Inf. Serv.* **46**, 118–19.

LEE, G. L. G. (1972*b*).—The phenogenetics of a super-suppressor in *Drosophila melanogaster*. II. Suppression and back-mutation. *Aust. J. biol. Sci.* **26**, 189–99.

LEE, G. L. G., and FRASER, A. S. (1969).—Sex dimorphism and canalization in *Drosophila melanogaster*. *Aust. J. biol. Sci.* **22**, 1259–69.

LINDSLEY, D. L., and GRELL, E. H. (1968).—"Genetic Variations of *Drosophila melanogaster*." (Publs Carnegie Instn No. 627.)

MORGAN, T. H. (1929).—Data relating to six mutants of *Drosophila*. In "Contributions to the Genetics of *Drosophila simulans* and *Drosophila melanogaster*." (Publs Carnegie Instn No. 399. pp. 171–83.)

MORGAN, T. H., SCHULTZ, J., and CURRY, V. (1941).—*Yb. Carnegie Instn* **40**, 282–7.

NOVITSKI, E. (1963).—Note in new mutants section of *Drosophila Inf. Serv.* **38**, 51–3.

SCHULTZ, J., and BRIDGES, C. B. (1932).—Methods for distinguishing between duplications and specific suppressors. *Am. Nat.* **62**, 323–34.

STERN, C. (1956).—The genetic control of developmental competence and morphogenetic tissue interactions in genetic mosaics. *Wilhelm Roux Arch. EntwMech. Org.* Bd. 1495. pp. 1–25.