A HIGHLY SPECIFIC COMPLEMENTARY LETHAL SYSTEM IN DROSOPHILA MELANOGASTER

A. H. STURTEVANT

California Institute of Technology, Pasadena

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IN January, 1954, two prune-3[1] females (*Drosophila melanogaster*) were mated to three males from the *S/E-S* stock. There resulted 339 females (all, as expected, pn^+), and one bobbed male that was also pn^+ (it was later found that the *S/E-S* stock carried *bb*, so this male was clearly a nondisjunctional exception). The absence of the class of pn^3 males was wholly unexpected, since the usual result would be that such males would be as numerous as their pn^+ sisters. Several possible interpretations were tested, but the only one that turned out to be adequate was that there is a dominant autosomal gene (now called "Prune-killer", symbol *K-pn*) in the *S/E-S* stock, that kills all prune flies but is without effect on pn^+. Later experiments have shown that the *S/E-S* stock is homozygous for *K-pn*; rather extensive tests have failed to show its presence in any other stock—both mutant stocks and wild strains from widely scattered localities having been tested. It has also been found that *K-pn* is equally effective in killing pn^1, pn^2, or pn^3—the only independently arisen alleles at present available for study—and that *pn* females are killed as effectively as are *pn* males. No other effects of *K-pn* have been found. It has no detected effect on the phenotype in any combination tried; in particular, it has no modifying effect on the color of the eyes in any of the numerous mutant eye-colors with which it has been tried.

The experiments leading to these and other conclusions may now be outlined.

GENETIC TESTS

Seventeen females from the original culture were tested. One of them was shown, by further testing, to carry an ordinary sex-linked lethal. Of the others, 8 were mated to *pn* males: they produced 745 + ♀♀, 393 *pn* ♀♀, 723 + ♂♂, 351 *pn* ♂♂—i.e., about 2 + :1 *pn* among both sons and daughters. Seven females were mated to males from the *S/E-S* stock, and produced (disregarding S): 756 + ♀♀, 414 + ♂♂—i.e., the expected *pn* sons were absent. The remaining female was mated to an unrelated + male, and produced: 125 + ♀♀, 60 + ♂♂, 34 *pn* ♂♂—i.e., half her *pn* sons were missing.

These results were sufficient to indicate that a dominant autosomal gene was involved; it was also apparent that this gene could not be closely linked to *S*, since in the above tests some of the females used were *S*/+, and there was no obvious deviation from the expected proportions for *S* and S^+ among their offspring. However, *S* is near one end of the second chromosome, and is therefore not an efficient marker for that chromosome in heterozygous females. Accordingly a test was made that depended on the absence of crossing over in male Drosophila.

[1] The names of mutants and the symbols for the genes concerned are the standard ones used in Drosophila. For descriptions, loci, etc. see BRIDGES-BREHME 1944.

The uniformity of the *S/E-S* stock was first tested. Twelve matings (including the original one that led to the present study) were made of the type *pn*^{3} ♀ × *S/E-S* ♂. There resulted 1629 + ♀♀, 0 *pn* ♂♂, 4 *bb* ♂♂ (nondisjunctional and XO). Two matings of *pn*^{1} ♀ × *S/E-S* ♂ gave 198 + ♀♀, 0 ♂♂; one *pn*^{2} ♀ × *S/E-S* ♂ gave 121 + ♀♀, 0 ♂♂; one *pn*^{1}/*pn*^{3} ♀ × *S/E-S* ♂ gave 198 + ♀♀, 0 ♂♂. These tests, plus those listed above from matings to F_1 females, show that at least 23 males (probably more, since more than one male was used in most cultures) from the *S/E-S* stock were unable to produce *pn* offspring. It has therefore been assumed that this stock was homozygous for the gene concerned—and all experiments designed on this assumption have given results consistent with it.

The *S/E-S* stock was mated to males carrying the dominants *Pin* (chromosome II) and *Ubx* (chromosome III—formerly called *bx*^{D}), and F_1 *Pin*; *Ubx* males were mated to *pn*^{3} females. There resulted 227 *pn*$^+$ ♀♀, 92 *pn* ♂♂—i.e., half of the *pn* offspring were absent. Further, the 92 *pn* males were classified for the dominant markers: there were 50 *Pin Ubx*, 42 *Ubx*. *K-pn* showed no recombination with *Ubx*, and is therefore in the third chromosome.

The determination of the locus of *K-pn* in the third chromosome has been somewhat more troublesome. The first test was with the two dominants Lyra (*Ly*, locus 40.5) and Stubble (*Sb*, locus 58.2). Males *pn*^{3}; *Ly Sb*/+ were mated to females from the *S/E-S* stock. The resulting females of the constitution +/*pn*^{3}; *S*/+; *Ly Sb*/*K-pn* were mated to *pn*^{3} males. There resulted (disregarding *S*) 525 *pn*$^+$, and the following 252 *pn*: *Ly Sb* 117, *Ly* 18, *Sb* 24, +93. These data show only that *K-pn* is not between *Ly* and *Sb*, nor is it near either one—i.e., it is near one end or the other of the chromosome.

This result showed that it would be necessary to use multiple recessive stocks to locate *K-pn*, and for this reason it was desirable to establish a strain of it free of *S* (which interferes with the classification of some third chromosome markers) and of *E-S* (which carries an inversion in II that might affect crossing over in III). Accordingly, from the test just described, *pn*$^+$; *Sb* males were selected and tested individually by *pn*$^+$/*pn* females. One such male gave no *pn*; *Sb* sons. His *pn*$^+$; *Sb* sons were mated to females from the *S/E-S* stock, and the *S*; *Sb* offspring were mated together. These flies were of the composition *S*/+; *Sb K-pn*/ *K-pn*. From their offspring *S*$^+$ were selected, and a stock maintained that has in fact been shown to be homozygous for *K-pn*. *Sb* has been maintained in it by selection, since for some experiments it is useful to have the *K-pn* chromosome marked by a dominant.

Two methods have been used to locate *K-pn*. One is to mate *Sb K-pn*/ *K-pn* to a multiple recessive, mate the F_1 *Sb* females to the same multiple recessive, and test various types of *Sb*-carrying crossover sons individually by *pn* females. In case the tested crossover carries *K-pn*, no *pn*; *Sb* sons are produced, whereas if the crossover does not carry *K-pn* the *pn* sons are *Sb* and *Sb*$^+$ in equal numbers. Tests of this sort soon showed that *K-pn* lies near the right end of the chromosome, and accordingly the second method was used to get a more precise location.

This method is like that used in the *Ly Sb* test described above, and gives a direct testcross for *K-pn*; but it requires the production of somewhat more complex stocks. Several such tests were made; two of them may be recorded here, since the others gave no additional essential information.

Females of the composition $\frac{+}{pn}$; $\frac{Sb \quad ca \; K\text{-}pn}{e^s}$ were mated to e^s *ca* males. The *pn* offspring were of the following classes (numbers in parentheses indicate the intervals in which crossing over occurred): (0) e^s, 88; (1) *Sb* e^s, 14: (2) *Sb*, 38; (3) *Sb ca*, 4; (1, 2) +, 2; total, 146. It follows that *K-pn* lies to the right of *ca*. There is only one marker gene now available that lies further to the right than *ca*—namely, brevis (*bv*). Accordingly, two tests were carried out: (A) $\frac{+}{pn}$; $\frac{ca \quad K\text{-}pn}{bv}$ and (B) $\frac{+}{pn}$; $\frac{\quad K\text{-}pn}{ca \; bv}$, both kinds of females being mated to *ca bv* males—which in most cases were also *pn*. The following results were obtained:

	pn^+ offspring		*pn* offspring			
	0	1	0	1	2	1, 2
A	971	25	204 (*bv*)	3 (*ca bv*)	0 (*ca*)	0 (+)
B	620	8	443 (*ca bv*)	9 (*bv*)	1 (+)	0 (*ca*)

(The relative numbers of pn^+ and *pn* here are not significant, since in some cultures the fathers were pn^+ and in others they were *pn*—and also the pn^+ were not classified for *ca* and *bv* in all cultures.)

These data give the locus of *K-pn* as 0.2 units to the right of *bv*. The latter is listed by BRIDGES-BREHME (1944) at 104.3, with only one locus—*M(3)g*—to the right of it, at 106.2. This latter mutant has been lost, and in view of its slight phenotypic effect, dependence on a linked enhancer, and phenotypic resemblance to brevis, one may question the accuracy of the determination of its locus. Brevis is listed as 3.6 units from *ca*; the present data give $100 \times 45/2284 = 2.0$ for the recombination percentage. In view of BRIDGES' custom of using maximum observed recombination values for map distances, this is a reasonable agreement with expectation, and the locus of *K-pn* may be put at 104.5, making it the rightmost locus now available for study in the third chromosome.

DR. E. B. LEWIS has kindly examined the salivary gland chromosomes of *K-pn* larvae; he finds the right end of chromosome III normal in appearance.

Various kinds of tests other than those already described have been carried out, and have confirmed the conclusions there drawn. Attached-X *pn* females × *K-pn*/*K-pn* males gave pn^+ sons and a few pn^+ superfemales; attached-X pn^+/pn^+; *K-pn*/*K-pn* females × *pn* males gave pn^+ daughters and no sons. The first of these two results shows that *pn*/*pn*/pn^+; *K-pn*/+ survives—a conclusion confirmed by the use of two different short duplicating fragments containing pn^+. In this way it is possible to obtain *pn*/pn^+; *K-pn*/+ males, and *pn*/*pn*/pn^+; *K-pn*/+ females, that are viable and fertile, since the pn^+ is contained in a small duplicating fragment that does not seriously affect viability or fertility. (The duplications used were *Dp (1;f)* X^{c2} and *Dp (1;4)*^{m5}—see BRIDGES-BREHME 1944).

Properties of K-pn

From the mating of *pn/pn* ♀♀ × *K-pn/K-pn* ♂♂, all the regular daughters are viable and all the regular sons die. Examination of such cultures, when the parents are transferred daily, shows that the eggs hatch and the larvae appear normal. However, the male larvae (*pn*; *K-pn*) do not develop beyond the second instar. They appear superficially normal until the time when their sisters molt at the end of the second instar; at this time they usually crawl out of the food, and all of them die without undergoing the molt. No detailed study of them has been made, but no structural abnormality has been observed.

It is clear from some of the tests described above that *pn* females carrying *K-pn*, and *pn* males that are homozygous for *K-pn*, also die; no attempt has been made to determine the time of death for these classes.

It was hoped that mosaics might be obtained that would contain parts that were *pn*; *K-pn* in composition, in order to determine the phenotype of specific regions of that composition. Two different tests were carried out, using *y pn* so that yellow bristles and hairs would serve as an index of the presence of *pn*, and using ca^{nd} (LEWIS and GENCARELLA 1952) as a source of mosaicism. It does not seem necessary to present the details of these experiments, since they were not wholly conclusive. There was a deficiency of mosaics carrying *K-pn* and with male parts *pn* in composition—but it is not certain that this deficiency was statistically significant. All such mosaics obtained had the yellow (i.e., male and genetically *pn*) parts confined to the thorax and abdomen—but again it is not certain (though it is probable) that the absence of *pn* eye-color was significant. In any case, the experiments did not answer the question of the possible effect of *K-pn* on eye color.

There is one reason for suspecting that *K-pn* may be related to pigment formation: the *pn*; *K-pn* larvae, that die at the end of the second instar, have been observed to have slightly paler Malpighian tubes than control *pn* larvae at the same age. However, it may well be that this is a symptom of approaching death, rather than a specific effect of *K-pn* on pigment synthesis.

K-pn/+ has been combined with a wide variety of eye-colors other than *pn*, and in no case has it been found to have any modifying effect on them. The list tried includes, among others: *v*, *cn*, *st* (which remove the brown pigment); *bw* (which removes the red pigment); w^a, w^e, zeste (which are known to be very susceptible to modifiers); *ca* (which lies near *K-pn*); and chocolate, *se*, and *pd* (which somewhat resemble *pn*). In the latter case, BRIDGES-BREHME (1944) described a special modifier of *pd* (purpleoid), called Purpleoider (*Pdr*) which has effects on *pd* somewhat similar to the effects of *K-pn* on *pn*. Tests have shown that *K-pn* does not affect *pd*, and that *Pdr* does not affect *pn*.

If *K-pn* kills *pn* through an action on some substance present in *pn* but not in other flies, it seemed possible that this hypothetical substance might be involved in the production of eye-pigment, and that it might be absent in white-eyed flies. Accordingly *pn w*, and *pn*; *cn bw* (both with eyes without pigment) were tried; *K-pn* was fully effective in killing both types. Such a result was perhaps to have been expected, since the *pn*; *K-pn* larvae die at a stage where the synthesis of eye-

pigment has presumably not yet begun—although the pigment of the Malpighian tubes (also absent in *w* and in *cn bw*) is already present in *pn* larvae at that stage.

DISCUSSION

The case of *pd* and *Pdr*, briefly referred to above, is somewhat like the present one. It differs in that each mutant gene has an effect on the color of the eyes in the absence of the other one, and in that the lethal effect is found only when both genes are homozygous (BRIDGES-BREHME 1944). No detailed account has ever been published.

Another analogous situation is that found with crossveinless (*cv*) and fused (*fu*) in *Drosophila simulans* (STURTEVANT 1929). Fused has reduced viability, and this is greatly exaggerated in *cv fu*, which has a phenotype that appears to be the simple sum of *cv* and *fu*, but has such a low viability that it rarely survives to the adult condition.

Perhaps a closer analogy is provided by the "synthetic lethals" described for *D. pseudoobscura* by DOBZHANSKY (1946). In this case it was found that two second chromosomes, neither of which had a lethal effect, might produce a lethal chromosome by crossing over—presumably through the bringing together of complementary recessive genes.

The situation is also similar to the well-known examples of genes that are lethal or cause tumors in species hybrids but not in the species in which they are normally found. (See, for example, HOLLINGSHEAD 1930 and GORDON 1931.)

There are other more or less similar examples scattered through the literature—cases where genes, that themselves may or may not have observed phenotypic effects, produce unexpectedly great effects on phenotypes conditioned by other genes. One has only to refer to the extensive literature on "enhancers" or "specific intensifiers" on the one hand, or "suppressors" on the other hand, to locate numerous examples.

K-pn may be thought of as an extreme "enhancer"—though it is not known whether it has any effect on the most obvious phenotypic effect of *pn* (the eye color); and if *pn* has any effect on viability (as it presumably does), it is not one that is easily detected in ordinary experiments.

It may be that specific "killers" are more frequent than would be inferred from the previous failure to recover any as effective and as specific as *K-pn*. This will become evident if it is recognized that the present case owes its discovery to the facts that *pn* is a sex-linked recessive and that *K-pn* is an autosomal dominant. The analysis would probably have failed if it had not happened that the *S/E-S* stock was homozygous for *K-pn*. It seems probable that these rather special requirements for discovery and analysis will not be met with in many cases—i.e., it is probable that recessive "killers" exist, that autosomal mutants are subject to such killing, and that most "killers" exist in heterozygous form in the stocks where they occur.

There is one use to which the present system may be put, in the design of experiments. In the two matings

$$pn\ ♀ \times K\text{-}pn/\ K\text{-}pn\ ♂$$

$$\text{attached-X}\ K\text{-}pn/\ K\text{-}pn\ ♀ \times pn\ ♂$$

no males are produced except by nondisjunction (in the first case) or detachment of attached-X's (in the second case). These two matings therefore give convenient and efficient methods of producing large numbers of all-female cultures—and therefore of necessarily virgin females. For some types of experiments the procedure may be found useful.

One possible hypothesis is that *pn* leads to the production (or accumulation) of some substance that is absent, or present only in small amounts, in pn^+ flies, and that *K-pn* converts this substance to a different and toxic substance. Such a hypothesis at least suggests experiments to be carried out, and offers some hope of relating the phenomena to the reactions concerned in the synthesis of pigments.

SUMMARY

1. The *S/E-S* stock was found to be homozygous for a gene called "Prune-killer" (symbol, *K-pn*), that is located at 104.5 in chromosome III.

2. *K-pn* has only one known effect: it causes the death of all prune flies that carry it, this effect being dominant.

3. This lethal effect of *pn*; *K-pn* occurs with pn^1, pn^2, or pn^3—the only separately arisen *pn* mutants available.

4. In the only case studied—males that are *pn*; *K-pn*—death occurs at the end of the second larval instar, and no gross abnormality was detected in the dying larvae.

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