A GENETIC ANALYSIS OF THE KILLER-PRUNE (*K-pn*) LOCUS OF *DROSOPHILA MELANOGASTER*

ELIEZER LIFSCHYTZ AND RAPHAEL FALK

Laboratory of Genetics, The Hebrew University, Jerusalem, Israel

Received January 21, 1969

IN a previous paper (LIFSCHYTZ and FALK 1969b) we showed that by using the specific interaction *pn*—*K-pn* (prune—Killer-prune) we could construct an efficient screening system in which the products of reversions in the *pn* and *K-pn* loci were the only viable male-progeny. The few female progeny that survived served for estimating the number of tested zygotes.

K-pn is a dominant mutant, interacting with *pn* to give a highly specific and most efficient lethal effect; it is located at the extreme right end of chromosome *3* of *Drosophila melanogaster* (STURTEVANT 1956). It is impossible to cancel the interaction by introducing into the genotype, besides *pn* and *K-pn*, additional markers which affect pteridine metabolism (STURTEVANT 1956).

Little is known about the mechanism of action of dominant mutants. The fact that *K-pn* is a "conditional dominant lethal" offers an opportunity to study the nature of such a gene. In the present study we tried to clarify the properties of the *K-pn* locus by genetic analysis, i.e. by an analysis of the spontaneous and induced reversions of the *K-pn* effect.

MATERIALS AND METHODS

Radiation was given with a G E-Maximar-100 machine at 100 kvp, 10 ma and a target distance of 10 cm with a 1 mm Al-filter.

Ethylmethane sulfonate (EMS) was fed to adult Drosophila flies for 24 hrs according to ALDERSON's (1965) method at an EMS concentration of 0.2% in 2% sucrose solution. The stocks that were used and their construction were described in detail in our previous paper (LIFSCHYTZ and FALK 1969b).

RESULTS

The products expected in the *pn*—*K-pn* selective system have already been described (LIFSCHYTZ and FALK 1969b). The crucial mating for obtaining rare events at the *pn* and *K-pn* loci is:

$$(1)\qquad \frac{y^{2}\ pn^{x}\ cv\quad l^{B57}\ l^{+}}{y^{+}\ pn^{y}\ cv^{+}\ l^{+}\quad l^{DES}}\ \frac{ca^{+}\ K\text{-}pn^{+}}{ca^{+}\ K\text{-}pn^{+}} \times \frac{v\ g\ l^{AA33}\ l^{3DES}}{Y\cdot mal^{+}}\ \frac{ca\ \ K\text{-}pn}{ca\ \ K\text{-}pn}$$

The rare male progeny with prune phenotype were the object of the present analysis. The *pn*-males could survive as a result of:

a) Incomplete penetrance of the *pn*—*K-pn* interaction. b) A dominant suppressor-mutation of the *K-pn* effect. c) A back mutation at the *pn*-locus that affected only the specific interaction with *K-pn*, but not the prune eye colour. d) A reversion at the *K-pn* locus.

TABLE 1

K-pn—*revertants obtained from various matings*

Treatment	Number of recombinant females	Number of *K-pn* revertants	Females/ revertant	Estimated frequency of reversion
spontaneous	6080	2	3040	1.7×10^{-6}
spontaneous	6142	2	3070	1.7×10^{-6}
X rays	734	14 (+2)	52	1×10^{-4}
EMS	263	6	44	1.1×10^{-4}
EMS	330	9	37	1.4×10^{-4}

In two separate experiments (Table 1, see also lines 1 and 2 in Table 2 of LIFSCHYTZ and FALK 1969b, see there also for calculation of frequency) four *pn* males were recovered, at a rate of one male per 3000 females, i.e. at a calculated frequency of 1.7×10^{-6}. Three were marked with y^2 and *cv*, the fourth was +. Each male was numbered and given the prefix "RK" (Reversed Killer). The males were mated individually with *pn²/FM6*; *ca K-pn* females. Only three males were fertile and the results of the analysis were similar for all three.

Since half the progeny—males and females—were claret it can be concluded that one of the chromosomes of the male carried the paternal third chromosome, originally *ca K-pn*.

The absence of any *prune* progeny indicated that the tested male did not carry a dominant suppressor of *K-pn* on the *X* chromosome or on the autosomes (thus excluding alternative b).

Claret sons of the RK males were mated with females homozygous for various *pn* alleles. In all matings the ratio of *pn* male-progeny to females was 1:2. This indicated that the survival of the original RK male was neither a result of incomplete penetrance of the *pn—K-pn* interaction (alternative a), nor a result of a mutation in the *pn* locus that reversed only the *K-pn* sensitivity (alternative c).

The presence of the proper lethal markers on the exceptional male was shown by appropriate tests. The conclusion was reached that the event leading to the appearance of a *pn*-male was a mutation in the chromosome that carried the *K-pn* locus, most probably at this locus (alternative d). The genotype of the male was thus:

$$(y^2)\ pn^x\ (cv)/Y\,mal^+\ ;\ ca\,K\text{-}pn^{+RK}\ /\ ca^+\ K\text{-}pn^+$$

The test-mating of the RK males may be reconstructed as shown in mating scheme (2).

The $K\text{-}pn^{+RK}$ mutants will be referred to only by their RK designation, for brevity.

Two types of events could occur at the *K-pn* locus, giving the original *K-pn* mutant:—(1) A neomorph or an antimorph mutation (MULLER 1932). (2) A hypomorph or an amorph mutation.

According to the first possibility $K\text{-}pn^+$ could be also an amorph. According to the second possibility $K\text{-}pn^+$ cannot be an amorph. We would be able to distinguish between the two possibilities if we knew the nature of the RK-revertants. If RK was a deficiency the second possibility is excluded.

MATING SCHEME 2

For the test of revertants at the *K-pn* locus

$$\frac{pn^2}{y^{34}\ sc^8\ dm\ B}\ \frac{ca\ K\text{-}pn}{ca\ K\text{-}pn} \times \frac{y^2\ pn^x\ cv\ l^x}{/\ mal+}\ \frac{ca\ \ K\text{-}pn+^{RK}}{/\ ca+\ K\text{-}pn} +$$

paternal gametes	maternal gametes: pn^2; *ca K-pn*	maternal gametes: $y^{34}\ sc^8\ dm\ B$; *ca K-pn*
$y^2\ pn^x\ cv\ l^x$; *ca K-pn*$+^{RK}$	♀ prune-claret died	♀ yellow, Bar-claret
$y^2\ pn^x\ cv\ l^x$; *ca+ K-pn+*	♂ prune died	♂ yellow, Bar
$Y \cdot mal+$; *ca K-pn*$+^{RK}$	♂ prune-claret died	♂ yellow, Bar-claret
$Y \cdot mal+$; *ca+ K-pn+*	♂ prune died	♂ yellow, Bar

The *RK* chromosomes were isolated from the sons of the original males with the aid of *D* and *Sb* balancer-chromosomes. Since no fly homozygous for the *ca RK* chromosome emerged in the progeny of any of the three *pn* males it was concluded that these chromosomes carried recessive lethal mutants. They were maintained as balanced stocks over the *D* or *Sb* balancer-chromosomes.

Since all three *RK* chromosomes carried recessive lethals it was suspected that these were small deficiencies. X rays generally induce deficiencies in post-meiotic sperm (LIFSCHYTZ and FALK 1968), in contrast to EMS that induces a high proportion of "point mutations" (LIFSCHYTZ and FALK 1969a). In order to test whether reversions of *K-pn* were deficiencies and whether reversions without associated lethal effects might be obtained, induction of RK mutations with X rays and EMS was tried. One- to four-day-old males of the genotype $v\ g\ l^{AAss}\ l^{sDES}$/ $Y \cdot mal+$; *ca K-pn*, were irradiated with 3000R and then mated for four days to the proper females (see scheme (1)). 734 females emerged in 375 culture bottles, representing approximately 1.5×10^5 zygotes (Table 1).

In 14 bottles single *pn* males were recovered. In only one of the bottles two *pn* males were found. Eight of the males were y^2 *pn cv*, seven were only *pn* and one was *y pn*. The males were given the prefix "RKX". They were tested like the previous ones. Ten of the males were fertile. All carried reverse mutations of *K-pn* on their third chromosome and every one of the *RKX*-chromosomes carried also a recessive lethal. Six of the chromosomes were maintained balanced over the *Sb*-chromosome.

Of the two males that emerged in the same bottle, only one was fertile and proved to be like the others. The fact that one of these males was not fertile may indicate that their origin was independent, yet we treated these two males as if they resulted from a single spontaneous premeiotic event. Excluding this pre-

sumed spontaneous premeiotic event, the frequency of the X-ray induced reversions was one *pn* male per 52 females, or approximately 1×10^{-4}.

In two separate experiments males of the appropriate genotype were treated with EMS and then mated for five days with proper females according to scheme (1). At the same time a small random sample of treated males was mated with females heterozygous for the balancer chromosome *FM6*[1]. 170 daughters were mated individually to males carrying a *Y·mal*$^+$ chromosome. The *X* chromosome of 82 (48%) was found to carry newly induced recessive lethals.

Fifteen prune males were found among the progeny of the matings, at a rate of one *pn* male per 40 females, or approximately a frequency of 1.2×10^{-4} (Table 1). The males were given the prefix "RKC". Twelve of the 15 males were fertile. The testing process was shortened this time by first mating the males with females of the stock *RKX-9/Sb*. All fertile males turned out to carry on their third chromosome a recessive lethal mutant, allelic to that of *RKX-9*. In an additional mating the presence of *ca* was confirmed. Eight of these chromosomes were established as stocks with *D* or *Sb* balancer-chromosomes.

More *RK*-chromosomes obtained from X-ray induction experiments were all found to be reversions at the *K-pn* locus associated with recessive lethality. This suggests that reversions of *K-pn* are always accompanied by a recessive lethal mutation at the *K-pn* locus.

To examine further whether the lethals were due to *different* events at or near the *K-pn* locus, complementation tests between lethals were performed. Among the various combinations between over 20 *RK*-chromosomes of different origin not a single case of complementation of the lethal effect was observed. It may, therefore, be inferred that the reversion of the *K-pn* effect was caused by a mutation at the *K-pn* locus itself and that it was always due to an inactivation of the locus. Furthermore, the function of the normal allele of *K-pn* appears to be essential for the development of the adult fly; this function was not lost when the *K-pn* mutation occurred.

DISCUSSION

The finding that all *RK*-chromosomes had recessive lethals and that all lethals were allelic indicated that all had at least one common lethal mutation, namely, that affecting the *K-pn* mutant to revert its specific interaction with *pn*.

Since *K-pn* itself is not a recessive lethal it is obvious that the site of the *pn*—*K-pn* interaction of the locus is not identical with a vital site. Point-mutations at the site of the specific interaction of the *K-pn* locus would not inevitably affect vital sites, while deficiencies of the locus would always affect both activities. This would suggest a lower efficiency of EMS in the induction of *RK*-mutants than that obtained with X rays (LIFSCHYTZ and FALK 1969a). Indeed, while X rays and EMS gave one *pn* male for each 52 and 40 females, respectively, the corresponding frequencies of recessive lethals induced in the *X*-chromosome were approximately 10% and 48%, respectively. In other words, X rays were about four times more efficient in the induction of *K-pn* reversions than EMS. This leads to the conclusion that *K-pn* revertants were deficiencies, comprising at least the *K-pn* locus.

These considerations exclude the possibility that the *K-pn* mutant was a hypo-

morph or an amorph allele of *K-pn*$^+$ and suggest its neomorphic character. Two assumptions concerning the nature of the *K-pn* mutant may be considered:

(a) The *K-pn*$^+$ gene (or its product) participates in two different reactions, the site of only one of which was changed in the *K-pn* mutation.—(b) The *K-pn* mutant is a partial duplication (with or without rearrangement) of the *K-pn*$^+$ gene, both genes were maintained in *K-pn* flies. The new *K-pn* gene is responsible for the *pn—K-pn* interaction, while the original *K-pn*$^+$ gene is responsible for some essential product.

The relatively high frequency of spontaneous and induced reversions could suggest that the original *K-pn* mutant was due to some duplication by a process of unequal recombination, in line with the second assumption. If this assumption was correct, some *RK*-chromosomes should be non-lethal, or at least should complement the lethal effects of other *RK*-chromosomes. Since this is not so, the conclusion that the *K-pn* mutant was due to a change at the *K-pn*$^+$ locus itself—probably by a point mutation—is called for.

The finding that all *K-pn* revertants were recessive lethals gave us a unique opportunity for the detection of recessive lethals already in F_1. Every fly that escaped death had a lethal mutation at the *K-pn* locus, while all zygotes with no lethal mutation died. The recessive lethals are all at a *given* locus and of a specific type of mutation, thus overcoming many of the difficulties when studying, without discrimination, heterogeneous mutants induced by different mutagens under varying conditions (STADLER 1954; AUERBACH 1966; AUERBACH and WOOLF 1960).

As our selective system detected only reversions at the *pn—K-pn* interaction site, we lost probably all point mutations that would produce recessive-lethality alone—this was apparently the source of the "differential specificity" of *K-pn* reversions towards the two mutagens (LIFSCHYTZ and FALK 1969a).

Recently Drosophila came again into focus as an appropriate organism for the study of genetic control mechanisms of differentiation. The usual approach is to choose a system which is favourable for biochemical analysis and then, to investigate its genetics. This study represents the opposite approach, where the system was selected primarily because of its interesting features from the genetic point of view. We hope that now enough clues are available to stimulate a biochemical study of the action of *pn* and *K-pn* in differentiation.

Note added in proof: A new *Y*-translocated chromosome—*Y·K-pn*$^+$—was constructed recently in our laboratory (FALK and SHAMAY). It contains the rightmost tip of the 3rd chromosome, including the *ca*$^+$ and *K-pn*$^+$ loci. Of the four *RK*'s tested with it so far, *Y·K-pn*$^+$ covered the lethal effect of two, while it did not cover the lethal effect of the remaining two—indicating that these were deletions extending even beyond *ca*$^+$.

We wish to thank Miss A. ITIN, Miss M. BROIT and Mrs. S. BAKER for their devoted help.

SUMMARY

In screening experiments for rare spontaneous events at the *pn* and *K-pn* loci, prune flies were detected at a frequency of 1.7×10^{-6}. The fertile flies were shown

to be due to genetic events at the *K-pn* locus. EMS was less efficient in the induction of such reversions of *K-pn* than were X rays. All revertants were recessive lethals and all these lethals were allelic. The revertants were presumed to be deletions of the *K-pn* locus. It was concluded that the *K-pn*$^+$ gene has a vital function which was not lost when the *K-pn* mutation occurred. The *K-pn* mutant was found to be neomorphic.

LITERATURE CITED

ALDERSON, T., 1965 Chemical induced delayed germinal mutations in Drosophila. Nature **207**: 164–167.

AUERBACH, C., 1966 The specificity of mutagens. Proc. Roy. Phys. Soc. London **29**: 1–10.

AUERBACH, C., and B. WOOLF, 1960 Alpha and beta loci in Drosophila. Genetics **45**: 1691–1703.

LIFSCHYTZ, E. and R. FALK, 1968 Fine structure analysis of a chromosome segment in *Drosophila melanogaster*. Analysis of X-ray induced lethals. Mutation Res. **6**: 235–244. —— 1969a Fine structure analysis of a chromosome segment in *Drosophila melanogaster*. Analysis of ethylmethanesulphonate induced lethals. Mutation Res. **8**: 147–155. —— 1969b A system for screening of rare events in genes of *Drosophila melanogaster*. Genetics **62**:343–352.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations. Proc. 6th Intern. Congr. Genet. **1**: 213–255.

STADLER, L. J., 1954 The gene. Science **120**: 811–819.

STURTEVANT, A. H., 1956 A highly specific complementary lethal system in *Drosophila melanogaster*. Genetics **41**: 118–123.