A SYSTEM FOR SCREENING OF RARE EVENTS IN GENES OF *DROSOPHILA MELANOGASTER*

ELIEZER LIFSCHYTZ AND RAPHAEL FALK

Laboratory of Genetics, The Hebrew University, Jerusalem, Israel

Received January 21, 1969

FINE structure analyses of genes in higher organisms lags behind that in microorganisms. This is mainly due to the difficulty in finding suitable selective systems with high resolution power which would enable the accumulation of a great number of the rare mutational and recombinational events that are essential for such a study.

Studies aimed at analysing gene structure in Drosophila were mainly concerned with genes in which multiple alleles could be easily discerned. It turned out that most of these genes were not single functional units but rather complex-loci systems (CARLSON 1959; GREEN 1964; LEWIS 1967). Whereas these studies illuminated the structural relations between functional units on the chromosome, they did not throw light on the fine structure of the smallest functional unit, the cistron. This was achieved, however, by CHOVNICK *et al.* (1962, 1964) who devised an ingenious selective technique which made possible the analysis of the rosy (*ry*) cistron and led to the presentation of a linear map of several separate mutational sites in this cistron.

When position pseudo-allelism (LEWIS 1967) occurs in a complex locus, recombination between cistrons might not be distinguishable from recombination within cistrons. A selective system for rare events which is based on a specific interaction between genes would, however, detect intracistronic changes rather than intercistronic changes. We endeavoured therefore to construct a genetic selective system for the analysis of the prune locus (*pn*, 1–0.8), which was based on its specific interaction with the Killer-prune mutant (*K-pn*, 3–102.9) in order to obtain more information on the structure of genes in *Drosophila melanogaster*.

Flies homozygous or hemizygous for *pn* die in the presence of one or two doses of *K-pn* (STURTEVANT 1956). The interaction is most effective and absolutely specific. No visible aberration at the *K-pn* region has been detected cytologically. Non-*pn*, *K-pn* flies have a wild-type phenotype and they are of normal viability. The physiological basis for the interaction is obscure.

In the present paper we describe the construction and application of a new genetic selective system based on the *pn–K-pn* interaction in combination with a system of independent sex linked recessive lethals.

The screening system—general considerations: It is easy to construct a system in which the *pn–K-pn* interaction serves to kill either all F_1 males or all F_1 females. Suitable matings are, for example:

(1) $\quad pn/pn \; ; \; +/+ \times +/Y \; ; \; K\text{-}pn/K\text{-}pn$

where all females heterozygous for *pn* are viable, and all males hemizygous for *pn* die.

(2) $pn : = ; +/+ \times +/Y$; *K-pn/K-pn*

where only males emerge in F_1 (see GLASSMAN 1959).

In all these cases recombinations and back-mutations at the *pn* locus and *K-pn* locus will be the only progeny of their sex; those at the *pn* locus have normal eye colour, while those at the *K-pn* locus have a *pn* phenotype.

Yet, with these procedures the culture bottles would still be crowded with progeny and the number of flies that need detailed examination would have been decreased only by a factor of two in comparison with a non-selective system. In order to increase the resolution power considerably—a system is needed where practically the only viable progeny will be those that are the results of rare events in the two loci *pn* and *K-pn*. This can be achieved by superimposing on the *pn–K-pn* system, a system of *X*-chromosome lethals that are covered in the males by marked *Y*-chromosomes, that is, by *Y*-chromosomes to which sections of normal *X*-chromosomes were translocated.

Mating (3) and its products in scheme A show the main elements of such a system:

(3) $pn^x\ l^1\ l^{2+}/pn^y\ l^{1+}\ l^2$; *K-pn*$^+$/*K-pn*$^+ \times pn^+\ l^1\ l^2/Y \cdot l^{1+}\ l^{2+}$; *K-pn/K-pn*.

SCHEME A

Possible products of mating (3): $pn^x\ l^1/pn^y\ l^2$; *K-pn*+/*K-pn*+ × $pn^+\ l^1\ l^2/Y \cdot l^{1+}\ l^{2+}$; *K-pn/K-pn*

maternal gametes	regular $pn^+\ l^1\ l^2$; *K-pn*	$Y \cdot l^{1+}l^{2+}$; *K-pn*	paternal gametes non-disjunction $\frac{pn^+\ l^1\ l^2}{Y \cdot l^{1+}\ l^{2+}}$; *K-pn*	0; *K-pn*	*K-pn* reversion $pn^+\ l^1\ l^2$; RK	$Y \cdot l^{1+}l^{2+}$; RK
regular						
$pn^x\ l^1\ l^{2+}$; *K-pn*+	die l	die K	♀ $\widetilde{+}$	die K	die l	♂ *pn*
$pn^y\ l^{1+}\ l^2$; *K-pn*+	die l	die K	♀ $\widetilde{+}$	die K	die l	♂ *pn*
pn-recombinants or reversions						
pn l^{1or2}; *K-pn*+	die l	die K	♀ $\widetilde{+}$	die K&l	die l	♂ $\widetilde{pn}$
pn+ l^{1or2}; *K-pn*+	die l	♂ $\widetilde{+}$	♀ $\widetilde{+}$	die l	die l	♂ $\widetilde{+}$
l recombinants						
$pn^x\ l^1\ \ l^2$; *K-pn*+	die l	die K	♀ $\widetilde{+}$	die K&l	die l	♂ $\widetilde{pn}$
$pn^y\ l^{1+}\ l^{2+}$; *K-pn*+	♀ +	die K	♀ $\widetilde{+}$	die K	♀ $\widetilde{+}$	♂ $\widetilde{pn}$
non-disjunction $pn^x\ l^1\ l^{2+}/pn^y\ l^{1+}\ l^2$;						
K-pn+	die x	die K	die x	die K	die x	♀ $\widetilde{pn}$
0; *K-pn*+	die l	die x	♂ $\widetilde{+}$	die x	die l	die x

□ — most important types
~ — phenotype
RK: Reversed Killer

causes of death:
K: *pn–K-pn* interaction
l: lethal mutation
x: sex chromosome aneuploidy

Most F_1 males die because of the *pn–K-pn* interaction. Most females are inviable because they are homozygous for at least one of the lethal mutants. The only viable females may be obtained from gametes in which recombination between the lethals or paternal non-disjunction had taken place. The only viable males arise as a consequence of recombination or back-mutation at the *pn* or *K-pn* loci.

Using the same principles one could recover the chromosomes that resulted from rare events at the *pn* and *K-pn* loci in the female progeny:

(4) $pn\ l^1\ l^{2+}/pn\ l^{1+}\ l^2$; $K\text{-}pn^+/K\text{-}pn^+ \times pn/Y \cdot pn^+$; $K\text{-}pn/K\text{-}pn$.

In this scheme, however, the products of paternal and maternal non-disjunction would also yield viable non-*pn* female progeny so that special markers must be introduced in order to recognize them. A system utilizing attached-*X* is not practical since it would have to face the technical difficulty of maintaining females heterozygous for the *pn*-alleles in repulsion. It could, however, be used for the detection of reversions of lethals (see GLASSMAN 1959).

The construction of the selective system: To start with we constructed a selective system based on mating (3). We intend to utilize the $w^+ \cdot Y$-chromosome for this system. This chromosome contains the normal allele of *pn*, therefore a *pn* mutation was induced in it to give a $pn^-w^+ \cdot Y$ chromosome. The difficulty of obtaining proper lethal mutants, which would not interfere with recombination in the *pn*-region (LIFSCHYTZ 1967), together with the trouble of constructing the $pn\ l^x$ chromosomes, led us however to abandon the $pn^-w^+ \cdot Y$-chromosome and to use the $Y \cdot ma\text{-}l^+$-chromosome instead.

Males carrying the $Y \cdot ma\text{-}l^+$-chromosome have excellent viability and fertility. The segment of the *X*-chromosome corresponding to that of the marked-*Y* is about 2 recombinational units long so that there is room for many lethals in it, among which a proper pair may be found. The segment corresponds to a region 65 recombinational units away from *pn*, so that the induced lethals would not interfere with recombination in *pn*, even if they were small aberrations. Furthermore, no difficulty should arise in the construction of the proper $pn\ l^x$-chromosomes.

The most proximal euchromatic segment of the $Y \cdot ma\text{-}l^+$-chromosome is present also in the $w^+ \cdot Y$-chromosome (and in the $pn^-w^+ \cdot Y$-chromosome) (LIFSCHYTZ and FALK 1968b). Some lethals would, therefore, be covered by both marked-*Y* chromosomes while others, near them, would be covered by the $Y \cdot ma\text{-}l^+$-chromosome only. A judicious choice of two lethals enabled us to incorporate in the scheme a preliminary step—mating (5), by which large numbers of virgins for the crucial mating (3) could be obtained "automatically":

(5) $pn^x\ l^1/FM6^l \times pn^y\ l^2/pn^-w^+ \cdot Y$

where: l^1 is covered only by $Y \cdot ma\text{-}l^+$

l^2 is covered by both $pn^-w^+ \cdot Y$ and by $Y \cdot ma\text{-}l^+$.

$FM6^l$ is the $y^{34}\ sc^s\ dm\ B$ balancer chromosome, in which a lethal was induced (see LINDSLEY and GRELL, 1968).

Recombination between the lethals could be used for estimating the number of zygotes per culture. Lethals were chosen so that the recombination frequency between them would yield few female progeny in every culture bottle. Since the

chances to get recombinational or mutational events in a given culture bottle are low, the presence of a few additional flies per culture could also facilitate the survival of the lonely relevant progeny.

For the construction of the system we chose the following three lethal mutants (for details on the lethals consult LIFSCHYTZ and FALK 1968b):

a) Lethal 3^{DES}, induced with diethyl-sulfate, is covered by both marked-*Y* chromosomes.

b) Lethal *B57*, induced with X rays, is a small deficiency. It is covered only by the $Y \cdot ma\text{-}l^+$-chromosome. The recombination frequency between 3^{DES} and *B57* is 1%. This value was, however, determined under conditions different from those of the selective system and must be taken only as an approximate value. In some experiments lethal *B57* was replaced by lethal *B275*—which was also covered by $Y \cdot ma\text{-}l^+$, but located distally to *B57*.

c) Lethal *AA33*, induced with diethyl-sulfate, is allelic to *B57*. Lethal *AA33* was used for the construction of the double lethal chromosome, but it could not be utilized as single lethal marker in the $pn\ l^x$-chromosome. Under low culture density conditions there were many "Durchbrenner" females (i.e., females in whom the lethal phenotype was not penetrant) which made the selective system inefficient. Females heterozygous for *B57*/*AA33* were not viable under any culture conditions.

SCHEME B

Mating scheme for the detection of rare events at the *pn* and *K-pn* loci.

(a)	$\frac{y^2\ pn^x\ cv\ l^{B57}\ l+}{FM6^1}$ $\frac{ca+\ K\text{-}pn+}{ca+\ K\text{-}pn+}$ / ×	$\frac{y+\ pn^y\ cv+\ l+\ l^{3DES}}{/\ \ pn^-w+\ \ /}$ $\frac{ca+\ K\text{-}pn+}{ca+\ K\text{-}pn+}$	
(b)	$\frac{y^2\ \ pn^x\ cv\ \ l^{B57}\ l+}{y+\ pn^y\ cv+\ l+\ l^{3DES}}$ $\frac{ca+\ K\text{-}pn+}{ca+\ K\text{-}pn+}$ ×	$\frac{v\ \ g\ \ l^{AA33}\ l^{3DES}}{/\ \ mal+\ \ /}$ $\frac{ca\ \ K\text{-}pn}{ca\ \ K\text{-}pn}$	
(1)	$\frac{y+\ pn\ \ cv+\ l+\ \ l+}{v\ \ g\ \ \ \ l^{AA33}\ l^{3DES}}$	$\frac{ca\ \ K\text{-}pn}{ca+\ K\text{-}pn+}$	recombinant between lethals
(2)	$\frac{(y)\ pn\ (cv)\ \ l^{B57\ \text{or}\ 3DES}}{v\ \ g\ \ \ \ l^{AA33}\ l^{3DES}}$ $\frac{}{/\ \ mal+\ \ /}$	$\frac{ca\ \ K\text{-}pn}{ca+\ K\text{-}pn+}$	paternal non-disjunction
(3)	$\frac{y^2\ pn+\ cv+\ l+\ l^{3DES}}{/\ mal+\ \ /}$	$\frac{ca\ \ K\text{-}pn}{ca+\ K\text{-}pn+}$	recombinants at *pn*
(4)	$\frac{y+\ pn+\ cv\ l^{B57}\ l+}{/\ mal+\ \ /}$	$\frac{ca\ \ K\text{-}pn}{ca+\ K\text{-}pn+}$	
(5)	$\frac{y^2\ pn+\ cv\ l^{B57}\ l+}{/\ mal+\ \ /}$	$\frac{ca\ \ K\text{-}pn}{ca+\ K\text{-}pn+}$	back-mutants at *pn*
(6)	$\frac{y+\ pn+\ cv+\ l+\ l^{3DES}}{/mal+\ \ /}$	$\frac{ca\ \ K\text{-}pn}{ca+\ K\text{-}pn+}$	
(7)	$\frac{y^2\ pn^x\ cv\ l^{B57}\ l+}{/\ mal+\ \ /}$	$\frac{ca\ \ K\text{-}pn+}{ca+\ K\text{-}pn+}$	back-mutants at *K-pn*
(8)	$\frac{y+\ pn^y\ cv+\ l+\ l^{3DES}}{/mal+\ \ /}$	$\frac{ca\ \ K\text{-}pn+}{ca+\ K\text{-}pn+}$	

In order to recognize the relevant chromosomes and to distinguish between the different events of recombination and backmutation the following additional markers were used: 1. Since *K-pn* has no visible phenotype by itself chromosome 3 with the *K-pn* mutant, carried always the recessive marker *ca* (claret, 3–100.7), closely linked to it. 2. The recessive markers *v* (vermilion, 1–33.0) and *g* (garnet, 1–44.4) were introduced into the double lethal *X*-chromosome in order to be able to identify, if necessary, the paternal chromosome. 3. y^2 (yellow^2, 1–0.0) and *cv* (crossveinless, 1–13.7) were used as outside markers in coupling with one of the tested *pn* chromosomes. The mating scheme which was finally adopted is described in Scheme B.

For the analysis of 10^6 zygotes in the final mating we had to run mating (a) in 50 culture bottles, so that we could collect easily 5000–7500 heterozygote *pn* virgins.

Under our culture conditions we found that by increasing the number of parents per culture up to 15 the number of recombinant daughters was also increased. With further increase of the number of parents there was no corresponding increase in the number of progeny. We used, therefore, as a rule, 15 females per culture bottle.

Parents mated thus in 350 bottles could be transferred once after 3–4 days to fresh culture bottles and again after another 3–4 days, yielding about the equivalent of 10^6 tested zygotes. In every bottle of this mating 3–10 females emerged. These were the products of recombination between lethals and of non-disjunction in the ratio of 7:1 (types (1) and (2) in Scheme B). Since the distance between the lethal markers was such that about 0.5% of the zygotes gave viable daughters, every 5 females represented 1000 tested zygotes (note that only half the recombinants between lethals were viable). Since, however, this conversion factor is inaccurate, it is safer to compare different experiments by the number of recombinant females.

Since the variance in the number of females per bottle was small it was enough to etherize and count flies in a sample of the bottles in order to estimate the total number of females. The remaining bottles were scored for the presence of rare males without etherizing the flies. We found no difficulties in detecting males, if they existed. The bottles were examined once on the 13th day after mating and a second time on the 17th day after mating.

Rare events in the pn *and* K-pn *loci*: The system was run several times in order to detect the rare events that could be obtained. In the preliminary runs the system was not yet perfected, there were no outside markers on the *pn*-chromosome in some tests, and *AA33* was used instead of *B57*. There was, e.g., no way of distinguishing back mutants from recombinants at the *pn* locus. For the final system we chose various combinations of alleles from several sources (Table 1). The combinations tested and the number of females and rare male progeny are given in Table 2.

In the first two matings neither recombinants at the *pn* locus were found, nor a reversion of *pn*. In each of two culture bottles in the first mating a male of the phenotype y^2 *pn cv* was obtained. One *pn* male and one y^2 *pn cv* male was obtained in each of two bottles in the second mating. They turned out to be back-

TABLE 1

pn *alleles tested in the screening-system and their origin*

pn^1	original *pn*-mutant.
pn^{FG}	spontaneous from a natural population (FALK, unpublished).
pn^{59j}	spontaneous? NARAYANAN and WEIR (1965).
pn^{AA1}	from a fractional mutant induced with diethylsulfate (LIFSCHYTZ).
pn^{MS2}	from a mutant induced with ethylmethane sulfonate (LIFSCHYTZ).

mutants at the *K-pn* locus, as will be discussed in the subsequent paper (LIFSCHYTZ and FALK 1969).

In the three following runs females with different combinations of *pn* alleles were tested for rare spontaneous events at the *pn* locus, while males were treated with various doses of X rays and ethylmethanesulfonate in order to obtain induced back mutants at the *K-pn* locus.

No case of recombination in *pn* was obtained in these matings. Apart from the *K-pn* reversions another type of rare males, with normal eyes and no exchange between the outside markers of *pn*, emerged for the first time in experiment 3. These were suspected to be back mutants at the *pn* locus. They were crossed to females of the genotype $pn^2/FM6$; *ca K-pn/ca K-pn*. Only one of the males was fertile. From the progeny test it was concluded that this fly was heterozygous for the *ca K-pn* chromosome. Further analysis showed that its *X*-chromosome carried a lethal that was covered by the $Y \cdot ma\text{-}l^+$-chromosome, its genotype was thus: $l^x/Y \cdot ma\text{-}l^+$; *ca K-pn/+*. The pn^+ phenotype could be due to one of the four following possibilities:

a) Recessive suppressor of *pn* on the *X* chromosome: *su-pn pn* $l^x/Y \cdot ma\text{-}l^+$; *ca K-pn/+*. b) Dominant suppressor of *pn* on an autosome: *pn* $l^x/Y \cdot ma\text{-}l^+$; *Su-pn/+*; *ca K-pn/+*. c) Dominant suppressor of *pn* on the *X* chromosome: *Su-pn pn* $l^x/Y \cdot ma\text{-}l^+$; *ca K-pn/+*. d) Back-mutant of *pn*: pn^x $l^x/Y \cdot ma\text{-}l^+$; *ca K-pn/+*.

The fact that among the testcross progeny pn^+ females were obtained excluded

TABLE 2

Genotypic combinations tested in screening system and progeny obtained

	Genotypic combination	No. of culture bottles	l^+ l^{2+} recombinant females	Estimated No. of zygotes	pn^+ recombinant males	pn^+-revertant males
1.	y^2 pn^{FG} *cv* l^{B57}/pn^{59j} l^{3DES}	1200	6080	1.2×10^6	—	—
2.	y^2 pn^{AA1} *cv* l^{B57}/pn^{59j} l^{3DES}	1170	6152	1.2×10^6	—	—
3.	y^2 pn^{AA1} *cv* l^{B275}/pn^1 l^{3DES}	1020	4163	0.8×10^6	—	1 y^2 pn^+ *cv* 1 pn^+
4.	y^2 pn^{AA1} *cv* l^{B57}/pn^{MS2} l^{3DES}	625	1985	0.4×10^6	—	2 pn^+
5.	y^2 pn^{MS2} *cv* l^{B57}/pn^1 l^{3DES}	705	2245	0.4×10^6	—	2 pn^+
6.	y^2 pn^1 *cv* l^{B57}/pn^1 l^{3DES}	900	3255	0.6×10^6	—	3 pn^+

possibility a. The absence of pn^+ males among the progeny in the testcross excluded possibility b. Further examination of the pn^+ chromosome of this fly gave no indication of the existence of a sex linked dominant suppressor which could recombine with *pn*. Unless a dominant suppressor was closely linked to *pn* it must be concluded that a back mutation of the *pn*-allele occurred according to possibility d.

Two non-prune males emerged among the progeny of experiment 4. Only one was fertile and was tested in the manner described above. The results were similar. Thus the genotypes of the two fertile rare males were:

$$pn^+\ l^z/Y \cdot ma\text{-}l^+;\ ca\ K\text{-}pn/+$$
$$y^2\ pn^{A A 1}+\ cv\ l^z/Y \cdot ma\text{-}l^+;\ ca\ K\text{-}pn/+.$$

In experiment 5 both pn^+ males were infertile.

In order to determine whether the appearance of these revertants was dependent on the heteroallelic combinations or whether they could also appear in homoallelic combinations, we tested the combination $y^2\ pn^1\ cv/pn^1$ (experiment 6). Only one of the three pn^+ males was fertile and behaved like the previous two males in test-crosses.

DISCUSSION

To date the most powerful screening system for rare events at genes of Drosophila was that of CHOVNICK *et al.* (1962) for the *ry* locus. The rosy system may be accommodated to the analysis of recombination of every gene next to which lethal mutations could be induced, while the present system is limited to cases where specific lethal interactions exist. This would not be too serious a limitation. STURTEVANT (1956) pointed out that there was no reason why such interactions between pairs of genes would not be found often. Indeed, the list of such interactions increases constantly (cf. LUCCHESI 1968). Another advantage of the rosy system is that recombinants would be recovered either as males or as females. These advantages of the rosy system are more than compensated for in the *pn–K-pn* system by its specific advantages:

1. No further selection among viable progeny is needed in order to identify the rare events (the rosy system calls for detailed examination of 10,000–50,000 progeny in order to detect an event that occurs at a frequency of 10^{-6} zygotes). Furthermore, rare events at two loci may be studied simultaneously. 2. The present system has a built-in device for the determination of the number of tested zygotes. 3. The system of selective lethals may be located independently of the examined loci. This facilitates the construction of the relevant stocks. The "automatic virgin" system is an additional advantage for experiments that must be performed on a very large scale.

In both systems, however, only one of the possible reciprocal products of recombination may be detected. This reduces the usefulness of the systems for the analysis of some events, such as "gene conversions." On the other hand, in contrast to the rosy system, ours makes possible the detection of back mutants at the studied loci.

It is significant that of the rare events that could be detected in our screening

system, only two types were recovered: revertants of the *pn* locus and revertants of the *K-pn* locus. The discussion on the *K-pn* revertants will be deferred to a later publication (LIFSCHYTZ and FALK 1969).

In those *pn*-revertants that could be analysed the possibility of a loosely linked suppressor has been eliminated. The possibility that the reversion was due to a closely linked suppressor or another mutation within *pn* was, however, not eliminated. Although it is impossible to determine accurately the frequency of reversions, even the most cautious estimates would not put it at less than 10^{-6} the order of magnitude of spontaneous forward mutations in many of the loci studied in Drosophila, including *pn* (SCHALET 1958). This is in line with our suggestion (LIFSCHYTZ and FALK 1968b) that the events that led to many of the so-called back mutants were probably minor aberrations and not genuine reversions of forward point-mutations. The fact that many of the pn^+ reversions were sterile is also in accord with this suggestion.

The appearance of rare flies other than the recombinants excluded the possibility that the recombinants were produced but were too weak to survive in the nearly empty bottles. The non-production of recombinants must thus be explained.

We chose for our analysis *pn* alleles from various sources (see Table 1). If recombination between *pn* alleles occurs—these would be a proper sample of mutants that should recombine, unless *pn* mutants were always aberrations.

The *pn* locus is in a chromosome region where recombination is rare (LEFEVRE and MOORE 1968). It could therefore be that recombination frequency in *pn* is much lower than in any other studied locus, including *ry*. Furthermore, it could always be argued that we happened to choose the wrong *pn* combinations—a negative result can never be accepted as final proof. Even in that case it may be concluded that if intra-genic recombination at the *pn* locus occurred, it was at least by one order of magnitude rarer than the frequency of reversions. NARAYANAN and WEIR's (1964) claim for recombination in the *pn* locus must be accepted with reservation since they had no outside markers to verify that their pn^+ flies were recombinants between pn^1 and pn^{59j}. We could not find any such recombinants in our experiment with the same alleles even when we repeated their own experimental procedure in a sample three times larger than theirs. We too obtained some pn^+ flies in preliminary experiments which were suspected to be recombinants (LIFSCHYTZ and FALK 1968a). In retrospect we think that all were revertants.

If the rare pn^+ flies were actually the products of recombinational events of the "gene conversion" type, we would expect at least some of these to be connected with recombination of the outer markers. This was not found. Furthermore, the frequency of the rare males that were obtained from females heterozygous for two different *pn* alleles was not higher than that obtained from females homozygous for the same *pn* alleles.

Theoretically it is conceivable that the *pn* locus is composed of two sub-units, one giving the prune phenotype, the other interacting with *K-pn*. If this were true a recombination restoring the prune phenotype should not necessarily always

restore the *K-pn* sensitive sub-unit as well. Such recombinants would not be recovered in the screening system. This would suggest also, however, the existence of *pn* progeny which were not sensitive to *K-pn* due to recombination in the other sub-unit. The fact that all *pn* flies recovered so far in the experiments were never due to events in or near the *pn* locus (see LIFSCHYTZ and FALK 1969) and the fact that none of the odd twenty *pn* alleles tested by us showed any separation of the prune eye colour from the *K-pn* sensitivity phenotype—makes such a subdivision of the *pn* locus a remote possibility. This was also supported by the finding that none of the tested genotypes pn^x/pn^y; *K-pn*/+ survived and that no induced *pn* mutants could be found in a *K-pn* genotype (LIFSCHYTZ and FALK 1969).

The fact that all *pn* mutants tested so far were similar in their eye-colour phenotype and were identical in their sensitivity to *K-pn* indicates that all mutants affect the same single cistron. For similar reasons *K-pn* must be considered as a single cistron (LIFSCHYTZ and FALK 1969).

With the progress of evolution there was a concomitant decrease in the rate of recombination per DNA base of the genome (PONTECORVO 1958). Presexual processes occurred at low frequencies in populations, so that the production of new alleles by intragenic recombination between different alleles was rare in any population of organisms. But with the establishment of regular sexuality the frequency of the production of new alleles by intragenic recombination in the populations would have been increased considerably. Recombination between already existing alleles was instrumental in speeding up evolution (MULLER 1932). Intragenic recombination producing new alleles, on the other hand, was, from the point of view of evolution, more like mutations than like intergenic recombination. Selection would thus act at a different intensity in regulating inter-genic and intra-genic recombination. The gene *pn* may be an extreme example of the outcome of such a process. The clustering of pseudo-alleles in many complex loci of Drosophila (e.g., *w*, *g*, *lz*, *dp*) into a small finite number of recombinational sites would indicate that in these complexes too, recombination is mainly inter-genic; intra-genic recombination being apparently still one or two orders of magnitude rarer.

We wish to thank MISS R. RONEN, MISS A. ITIN, MRS. S. BAKER, and MISS M. BROIT for their devoted help at various stages of this study.

SUMMARY

The construction of an efficient screening system for the detection of intracistronic recombinants and of back mutants in Drosophila is described. The system utilizes the specific lethal interaction of *pn* and *K-pn*, in combination with crisscross lethals in an *X* chromosome segment covered by a marked *Y* chromosome. No recombination between various *pn* alleles was obtained in over 4×10^6 zygotes. Revertants of *pn* and *K-pn* were obtained at frequencies similar to those of spontaneous forward mutations. The reasons for the absence of *pn*-recombinants was discussed. The possibility that intra-cistronic recombinants are rarer, per physical distance, than most recombinants in complex loci systems, which were due to inter-cistronic events, was considered.

LITERATURE CITED

CARLSON, E. A., 1959 Comparative genetics of complex loci. Quart. Rev. Biol. **34**: 33–67.

CHOVNICK, A., A. SCHALET, R. P. KERNAGHAN and J. TALSMA, 1962 The resolving power of genetic fine structure analysis in higher organisms as exemplified by Drosophila. Am. Naturalist **46**: 281–296.

CHOVNICK, A., A. SCHALET, R. P. KERNAGHAN and M. KRAUSS, 1964 The rosy cistron in *Drosophila melanogaster*: Genetic fine structure analysis. Genetics **50**: 1245–1259.

GLASSMAN, E., 1959 Methods for the selection of back mutations (and wild-type recombinants) at the prune locus of *D. melanogaster*. Drosophila Inform. Serv. **33**: 136.

GREEN, M. M., 1964 Genetic fine structure in Drosophila. Proc. 11th Intern. Congr. Genet. **2**: 37–49.

LEFEVRE, G., and L. B. MOORE, 1968 Recombination in regions adjacent to deletions in the *X*-chromosome of *Drosophila melanogaster*. Genetics **58**: 557–571.

LEWIS, E. B., 1967 Genes and gene complexes. Pp. 17–97, In: *Heritage from Mendel*. Edited by R. A. BRINK, The University of Wisconsin Press.

LIFSCHYTZ, E., 1967 Induced X-chromosome lethals covered by *Y.w+*. Drosophila Inform. Serv. **42**: 89.

LIFSCHYTZ, E., and R. FALK, 1968a A new system for fine structure analysis of genes in Drosophila. Drosophila Inform. Serv. **43**: 131. —— 1968b Fine structure analysis of a chromosome segment in *Drosophila melanogaster*. Analysis of X-ray induced lethals. Mutation Res. **6**: 235–244. ——1969 A specific analysis of the Killer-prune (*K-pn*) locus of Drosophila. Genetics **62**: 353–358.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of* Drosophila melanogaster. Carnegie Inst. Wash. Publ. 627.

LUCCHESI, J. C., 1968 Synthetic lethality and semi-lethality among functionally related mutants of *Drosophila melanogaster*. Genetics **59**: 37–44.

MULLER, H. J., 1932 Some genetic aspects of sex. Am. Naturalist **64**: 118–138.

NARAYANAN, Y. and J. A. WEIR, 1964 Paper chromatography of pteridines of prune and clot stocks of *Drosophila melanogaster*. Genetics **50**: 387–392.

PONTECORVO, G., 1958 *Trends in Genetic Analysis*. Columbia University Press.

SCHALET, A., 1958 A study of spontaneous visible mutations in *Drosophila melanogaster*. Proc. 10th Intern. Congr. Genetics **2**: 252.

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