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The action of the gene *prune* (*pn*) in *Drosophila melanogaster*

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1. INTRODUCTION

The gene *prune* (*pn*, 1–0·8) of *Drosophila melanogaster* affects the fly's eye colour; the mutants are easily detectable due to changes in their pteridine pattern. Another effect of *pn* is the interaction with the dominant mutant *K-pn*, located on the third chromosome; every *K-pn* fly homozygous or hemizygous for *pn* mutants dies (Sturtevant 1956). All known *pn* mutants interact with *K-pn*, this interaction is most efficient and is not changed even in the presence of other mutants in the genotype that affect pteridine pattern.

Pteridines found in the eyes of adult flies cannot be detected in early larval instars. They appear first in the late third larval instar and accumulate mainly during the pupal stage (Taira, 1961; Ziegler, 1961). The *pn–K-pn* interaction occurs at the second larval instar (Glassman, 1962; Lindsley & Grell, 1968) long before the eye pigments affected by *pn* may be detected. All pteridines of wild-type flies are present also in *pn* flies, but the amount of the red pigments (drosopterines) is reduced (Hadorn & Mitchell, 1951; Nolte, 1959; Narayanan & Weir, 1964), while that of the xanthopterine and sepiapterine is not affected or even slightly increased.

Mutants affecting pteridine pattern with pleiotropic effects, such as female sterility (*dor*), increased non-disjunction (ca^{ND}) or lethal interactions were described (see Counce, 1956; Lewis & Gencarella, 1952; Lucchesi, 1968). The availability of an efficient screening system for the selection of rare events at the *pn* locus (Lifschytz & Falk, 1969*a*) offered the opportunity to study the pleiotropic effects of *pn* on differentiation. The present study presents some observations on the nature of the gene *pn* and its interaction with *K-pn*.

2. MATERIALS AND METHODS

A description of the *Drosophila* mutants may be found in Lindsley & Grell (1968) and in our previous paper (Lifschytz & Falk, 1969*a*). Since for the present study the relevant fact about the $w^+.Y \neq 2$ chromosome was that it carried the pn^+ allele, this chromosome was designated here as $pn^+.Y$.

Irradiation was given with a Maximar-100 G.E. X-ray machine at 100 KVP, 5 mA with 1 mm Al filter.

Ethylmethanesulphonate (EMS) treatment was given to males 0–2 days old. The flies were kept for 24 h on a 0·2 % EMS, 2 % sucrose solution (see Lifschytz & Falk, 1969*b*).

Somatic recombination patches on the epidermis of the thorax and abdomen were observed under × 100 magnification, using transmitted light on whole-mount flies (Ronen, 1964).

Pteridines were extracted in 1% ammonia. Descending chromatography on Whatman no. 1 paper was run with propanol:1% ammonia, 70:10, for 12 h. Fluorescent materials were detected with u.v. lamps at a wavelengths of 250 and 360 mμ.

For uric acid determinations pupae were homogenized in NaOH at pH 10. The homogenate was treated for 3 min at 70 °C and centrifuged. Chloroform was added to precipitate proteins. The aqueous supernatant was concentrated and then separated by chromatography, as above. The uric acid was identified through a reference spot. It was eluted from the paper with 0·1 N-NaOH and its amount was determined in the spectrophotometer at 290 mμ. In another series uric acid was measured with the phosphotungstat reaction according to Caraway (1955).

For the extraction of hypoxanthine hot water was used in homogenization. The homogenate was heated to 80 °C for 3 min, centrifuged, proteins were precipitated with chloroform and the aqueous supernatant was concentrated and separated by chromatography. The hypoxanthine was eluted from the paper with 0·1 N-NaOH. Qualitative analysis was performed in the spectrophotometer at 255 mμ.

Crithidia fasciculata cultures were obtained from Dr I. Ziegler, Darmstadt. Culture techniques and bioassay techniques were according to Guttman (1964) with minor modifications according to I. Ziegler (personal communications).

3. RESULTS

(i) *Time of* pn–K-pn *interaction*

According to Glassman (1962) *pn–K-pn* males, but not females, die in the second larval instar. We decided to determine the age of death more precisely. Flies were mated in such a manner that either their daughters or their sons should die because of the *pn–K-pn* interaction:

(1) *pn/pn*; +/+ × +/Y; *caK-pn/caK-pn* for killing males,

(2) *pn/pn*; +/+ × *pn/pn*$^{+}$.Y; *caK-pn/caK-pn* for killing females.

Eggs were collected from Petri-dishes on which many females were allowed to lay eggs for 30–60 min. Counted batches of more or less synchronized larvae were transferred to fresh vials at given times after laying. If the batches of larvae were taken before the lethal interaction occurred only half the number of larvae developed into adults, while all larvae developed into adults if they were taken after the lethal interaction occurred. The results proved unequivocally that death occurred in *both* sexes in larvae 24–36 h after hatching from the eggs. The wide range was partly due to failure to synchronize more precisely the age of the larvae. It seems that death occurs at a precise age—around 30 h for females and around 36 h for males. An additional proof that females do not survive to the pupal stage was obtained when two non-complementing lethals (covered by

Y.*mal*$^+$) were introduced each into one of the *X*-chromosomes of the females in mating (2): not a single pupa was produced in these cultures. Identical results were obtained when the experiment was repeated with various *pn* alleles.

(ii) *Autonomy of the* pn–K-pn *interaction*

As noted by Beadle & Ephrussi (1936), *pn* is autonomous in transplantations of the imaginal disks of eyes, i.e. the presence of the wild-type pteridine pattern in the adjacent tissues did not help to overcome the defect of the *pn* mutant in the cells of the imaginal disk, neither did *pn* surrounding tissue interfere with the normal eye-colour development in a wild-type eye-disk.

The autonomy of the pleiotropic effect of *pn* in the *pn–K-pn* interaction was studied by us in three types of experiments where we tried to produce *pn–K-pn* tissue in *K-pn* non-*pn* flies. The first technique was the production of small patches of *pn–K-pn* tissue by induced somatic recombination (Stern, 1936). Larvae of the genotype *y ac sc pn sn*/+ ; +/*ca K-pn* were collected at the age of 70 h and irradiated with an X-ray dose of 1100 r. Exchange promixal to *sn* should give cells homozygous for *pn*. If such cells developed into epidermal tissue they would be recognizable by the *yellow* and *singed* phenotype. In 40 females 30 *y sn* spots, 3 *sn* spots and 1 *y* spot were detected. At least the presence of the *y sn* spots indicated that small *pn–K-pn* epidermal regions survived. It was, however, possible that these cells survived only because the interaction *pn–K-pn* failed to act in 70 h larvae, whereas it would have been lethal earlier at 30 h. The experiment was thus repeated with 24 h larvae. The results were identical to those obtained with older larvae, namely *y sn* spots were obtained. In these spots the *pn–K-pn* interaction was accordingly non-autonomous, either because there was no lethal interaction in cells of the epidermis or because the cells of the spot were supported by the non-lethal surrounding tissue.

It was thus desirable to obtain larger areas of *pn–K-pn* tissue, hoping that in some also internal organs of the flies would be involved. For this end gynandromorphs were produced with the aid of an X^{c2}, In(1)*w*^{vC}*f* chromosome that was frequently lost during mitosis. Gynandromorphs, one eye of which was *pn* were easily obtained from a mating *pn*/*pn* × X^{c2}, In (1)*w*^{vC}*f*/sc^8.Y. Up to half the females were transformed into gynandromorphs, many of them were half-body gynandromorphs.

In the mating X^{c2}, In(1)*w*^{vC}*f*/*y wlz*; +/+ × *pn*^{2}/*pn*$^+$.Y; *ca K-pn*/*ca K-pn* 450 females were obtained, among them three with half the abdomen—including external genitals—male-like. Another two had one *pn* eye. Large spots of *pn–K-pn* tissue could thus be non-autonomous. These spots were, however, rarer and smaller than those obtained in matings where no *pn–K-pn* interaction could occur. No typical half-body gynandromorphs were found.

In order to delineate more precisely the size of the non-autonomous patches the mating X^{c2}, In(1)*w*^{vC}*f*/*y wlz*; +/+ × *y pn sn*/*pn*$^+$.Y; *ca K-pn*/*ca K-pn* was performed. *y sn* spots should be detectable in the X^{cv}, In(1)*w*^{vC}*f*/*y pn sn*; *ca K-pn*/+ daughters. Of 422 females twenty-three had *y sn* spots. Most spots comprised

not more than half the thorax or half the abdomen. In two cases one eye was *pn* and its surrounding was *y sn*. There is no doubt that the non-autonomy may extend over rather extensive sections of the flies. The appearance of nearly half-body gynandromorphs indicated also that these non-autonomous sections may be established in the epidermis before the larvae were 30 h old; that is, these tissues survived the developmental stage of the lethal interaction.

In a third experimental technique we attempted to induce fractional *pn* mutations in a *K-pn* genotype. *ca K-pn* males were treated with 0·2 % ethylmethanesulphonate for 24 h and then mated to *pn sn* females. Among 10452 daughters there was one female with half the abdomen male-like and *sn* and another two with one male-like wing, these were cases of *X*-chromosome losses. Five females with whole-body *sn* mutations and eleven with fractional *sn* mutations were also obtained. Only a single female had one *pn* eye and another one had a *pn*-mosaic eye; both had normal female gonads and did not transmit a new *pn* mutation. The frequency of detectable induced *pn* mutations in this genotype was, however, lower than in the control experiments, where about one in 500 daughters was mosaic for a prune mutation which in many cases could be transmitted to the progeny. The non-autonomy of *pn–K-pn* spots was again confirmed. Furthermore, we did not obtain a *pn* mutant insensitive to the action of *K-pn*. Such an allele would have been detected in this experiment as a half-body (transmitable) *pn*-mosaic.

(iii) *The effect of the* pn *mutants*

The action of *pn* on the pattern of the pteridines may be either by interfering in the inter-conversion of already-existing pteridines or by interference in their synthesis. Beadle & Ephrussi (1936) demonstrated for a series of pteridine-mutants (*bw*, *Hn*, *pn*, *ras*, *se*) that transplanted eye-disks developed autonomously in regard to the eye-colour pattern. This would indicate that the synthesis of the pteridines occurs in the eyes themselves.

We constructed the genotypes *pn;se* and *pn;ry* and compared their pteridine patterns on paper chromatography under fluorescent light, with that of *pn* alone and that of *se* and *ry* by themselves. The amount of sepiapterine and biopterine in *pn;se* flies was at least ten times lower than that observed in *se* flies and approached the amount found in wild-type and *pn* flies. Thus the action of *pn* is not limited to the drosopterines. It apparently interferes with a step preceding the synthesis of drosopterines, sepiapterine as well as biopterine. The fact that the introduction of *pn* into *ry* caused only a further reduction in the amount of the drosopterines, without conspicuously affecting either the small amount of sepiapterine or any other pteridines, supports the notion that *pn* interferes in an early stage in pteridine synthesis.

It has been shown that the purine guanosine is the precursor of pteridines *in vivo* (Brenner-Holzach & Leuthardt, 1961, 1965; Levy, 1964; Watt, 1967). If pn^+ affects one of the early steps of conversion of guanosine into pteridine, a block in that step may cause accumulation of guanosine, and hence should also cause an increase in the products of its catabolic pathway, leading to uric acid. The amount

of uric acid in the early pupal stages, in wild-type and in *pn* flies, was compared. These measurements were performed up to 45 h after pupation; that is, during a time when the main acceleration in synthetic activity of pteridines took place. As can be seen in Tables 1 and 2, uric acid accumulated faster and to higher levels in *pn* than in wild-type pupae. In pupae 47 h old this amounted to an excess of up to 40 % in uric acid concentrations.

Table 1. *Amounts of uric acid and hypoxanthine (γ/mg wet weight) in prepupae and pupae of various genotypes (each value is the mean of two experiments with 15–30 pupae in each)*

	Prepupae (γ/mg)	48 h pupae (γ/mg)	Relative increase in pupae (%)
	Uric acid (chromatography)		
Berlin	1·54	3·51	100*
pn^{FG}	1·25	4·09	144
pn^{2}	1·65	4·43	135
	Uric acid (phosphotungstate)		
Berlin	1·64	3·99	100*
pn^{591}	1·87	5·64	160
	Hypoxanthine		
ry	0·37	1·49	100*
pn;ry	0·27	2·01	155

* Arbitrary-standard increase.

Table 2. *Amounts of uric acid in γ/mg wet weight in* sepia *and* prune;sepia *at various developmental stages (each value is the mean of two experiments with 12–20 animals in each)*

	sepia		*prune;sepia*	
	γ/mg	Increase (%)	γ/mg	Increase (%)
Larvae 80 h	1·17	100*	1·47	100*
Prepupae	1·64	135	2·62	178
Pupae 24 h	3·52	302	6·04	410

* Larvae 80 h taken as standard.

In order to verify that the excess accumulation of uric acid was due to an increase of the catabolic pathway of guanosine the test was repeated in *pn;ry* pupae. Since *ry* pupae lack the enzyme xanthinedehydrogenase, hypoxanthine instead of uric acid accumulates. As can be seen from the table, the amount of hypoxanthine was significantly higher in *pn;ry* pupae than in the *ry* pupae.

The bioassay method using the flagellate *Crithidia fasciculata* for detecting pteridines and their precursors allowed only very rough quantitative comparisons in our hands. It is, however, clear that no non-fluorescent Crithidia active (NFCA) material that was not present in wild-type pupae accumulated to a large extent in *pn* pupae.

4. DISCUSSION

The fact that large sectors of the epidermis in all parts of the body of the flies could survive in spite of the cells being of the genotype in which the *pn–K-pn* lethal interaction could occur shows that at least in the epidermis the effect is non-autonomous. Yet there was a shortage of such epidermal sectors, both in number and in size. This can be understood if it is assumed that as long as the 'lethal interaction genotype' does not affect some specific internal organs, the *pn–K-pn* epidermal sectors may survive. We assume that for certain specific cells of the organism the *pn–K-pn* interaction is autonomous. This assumption is also supported by reciprocal transplantation experiments between *pn* and *K-pn* flies. Grell (1958) concluded that the genes *pn* and *K-pn* must be in the same cells for the interaction to take place. The production of epidermal sectors is possible as long as the critical tissue is not affected; the epidermal sectors are thus allophenes, due to a mechanism of 'relational pleiotropism' (Hadorn, 1955; Stern & Tokunaga, 1968). For the cells of the eyes, the lethal interaction is not autonomous, however, since gynandromorphs with a *pn* eye and a surrounding *ysn* tissue in a *K-pn* background were obtained; the action of *pn* may be described as 'mosaic pleiotropism', where different tissues show different autophenes of the *pn* mutants, while in other tissues *pn* has no direct effect at all.

We excluded the possibility that the small sectors of *pn–K-pn* epidermis survived because they developed at a stage later than the sensitive phase of the lethal interaction (see Oster & Sobels, 1956), both by obtaining patches in larvae irradiated 24 h after hatching and by obtaining gynandromorphs of various sizes, including near-half ones.

At the end of her review on the pteridine pigments, Ziegler (1961) offers two alternative schemes for possible pathways of synthesis of eye-pteridines. Our evidence suggests that *pn* acts on an early step of such a pathway. The fact that *pn* mutants do not decrease the amounts of sepiapterine and biopterine, as compared with those in the wild-type flies, while they drastically decrease the amount in *pn;se* flies as compared with *se* flies, formally supports Ziegler's second alternative, i.e. that the pathway branches to the yellow pigment on the one hand and to the red pigments on the other. Sepiapterine is not an intermediary on the branch leading to the red pteridines, because if it were it would have been decreased in *pn* flies just as the red pigments were decreased. The interrelation between the change in sepiapterine and the red pigments in different mutants would accordingly be a function of the competitive ability of the two branching pathways.

The fact that none of the normal eye-pteridines is completely absent in *pn* mutants would indicate that *pn* is not a gene for one of the enzymes on a main pathway leading to the synthesis of these pteridines. It could, however, be involved in the rate of synthesis in a main pathway, either by producing some essential cofactor or by just being a regulatory gene.

In recent years it became obvious that the unconjugated pteridines are not merely inert metabolic end-products. Reduced pteridines were found to be potent

cofactors in such enzymic reactions as decarboxylation of phenylalanine (Kaufman, 1964), hydroxylation of steroids (Hagerman, 1964), and oxydation reactions of glycerol ethers (Tietz, Lindberg & Kennedy, 1964). On the other hand, pteridine is also a component of folic acid, and the pteridine-containing analogues of folic acid, such as aminopterine and ametopterine, are potent metabolic poisons and anti-mitotic agents. These agents were found to inhibit growth and development also in *Drosophila* (Goldsmith, 1953; Hinton, 1952).

That pteridine metabolism is involved in essential reactions *in vivo* is also indicated by effects of some mutants of *Drosophila*. Female sterility is a pleiotropic effect of some eye-colour mutants that effect pteridine metabolism (Counce, 1956). Lethal interaction between genes affecting pteridine patterns were summarized recently by Lucchessi (1968), and the effect of the mutant ca^{ND} on chromosome segration in meiosis and mitosis has been known for many years (Lewis & Gencarella, 1952). The mechanism for such disturbances may be due to a deterioration of an equilibrium between growth hormones and pteridines which presumably controls cell division in some insects (L'Helias, 1964). Harris & Forrest (1967) argue that some pteridines may actually regulate replication or transcription of DNA.

Since *pn* is involved in an early stage of the conversion of guanosine into pteridine it is a plausible speculation that precursors preceding the partial block accumulate in these mutants. The changed product of *K-pn* (Lifschytz & Falk, 1969*c*) could accordingly interact with one of these precursors to produce a poisonous material, related to the normal conjugated or unconjugated pteridine in some critical tissues of the larvae as soon as the pteridine pathway is triggered off.

SUMMARY

The gene *pn* was found to be active already in larvae 30 h old, long before eye-pteridines accumulated. At this developmental stage *pn* interacts with the gene *K-pn*. From studies with induced somatic recombination, gynandromorphs, and induced *pn* mutations, in a *K-pn* background it was concluded that the interaction *pn–K-pn* is non-autonomous in the epidermis, but in some internal organs it is apparently autonomous and causes the death of *pn–K-pn* flies. It is proposed that *pn* mutants interfere with an early step of the conversion of guanosine to pteridine, so that a deficiency of drosopterines, but not of the competing sepiapterine and biopterine, is caused. The accumulated precursor is presumably the substrate for a poisonous reaction triggered by *K-pn*.

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