PAPER CHROMATOGRAPHY OF PTERIDINES OF PRUNE AND CLOT STOCKS OF DROSOPHILA MELANOGASTER[1]

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WHEN a stock of *Drosophila melanogaster* with chromosome 2 marked by clot, *cl*, produced brown-eyed flies resembling clot but with the gene in the X chromosome, it was suspected that a translocation had occurred. However, genetic tests, including crosses to a newly acquired yellow prune stock (*y*, *pn*) showed that a mutation had occurred at the *pn* locus. The new allele, symbol pn^{59j}, is completely recessive to wild type, as are *pn* and *cl*, but in combination with clot produces a graded series of eye colors as follows: dark brown with genotypes pn^{59j}/pn^{59j}, *cl*/*cl*, and pn^{59j}/Y, *cl*/*cl*; intermediate brown with genotypes pn^{59j}/pn^{59j}, *cl*/+, and pn^{59j}/Y, *cl*/+; light brown with pn^{59j}/pn^{59j}, +/+ or +/+, *cl*/*cl*. Similar interactions were obtained from crosses using *y pn* and *cl* flies. Eye colors of living flies from stocks of *pn*, pn^{59j}, and *cl* are distinguishable.

By the time the mutant origin of pn^{59j} had been established, chromatographic studies were in progress and the emphasis had shifted to an analysis of biochemical differences among *pn*, the new allele pn^{59j} and *cl*. Not only are there significant pteridine differences between clot and the "alleles" at the prune locus, but also differences between *pn* and pn^{59j}.

Among 30,617 F_2 flies from reciprocal P_1 crosses between *pn* and pn^{59j} there were two with wild-type eye color, one male and one female. If *pn* and pn^{59j} are recombinationally separate loci (pseudoalleles) and if flies carrying the *cis* arrangement are not identifiable the estimate of recombination is .013 percent. Examination of the "region" of prune in salivary chromosomes failed to show a doublet.

METHODS

Stocks used were: (1) the original *cl* stock in which pn^{59j} arose; (2) a *y pn* stock obtained from the Institute for Cancer Research (Philadelphia); (3) pn^{59j}; and (4) attached-X yellow with *cl* added (used to determine genotypes of F_2 males).

Chromatograms were developed by the method described by HADORN and MITCHELL (1951) on 23 cm square sheets of Whatman No. 1 paper. Heads from 15 flies of the same sex (four to six days old), per sample, were mashed firmly on the paper with a glass rod, at a corner 1.5 cm from each edge. After the spots had dried, the sheets were placed in a cylindrical glass jar containing a shallow layer of the solvent mixture. Chromatograms were developed for approximately 8 hours in propanol:ammonia (two parts n-propanol to one part of 1 percent ammonium

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hydroxide), removed and allowed to dry at room temperature. Two-dimensional chromatograms were prepared by turning the sheets 90 degrees and exposing the samples to the water-saturated Collidine solvent for 8 hours. Chromatograms were developed and eluted in complete darkness since some of the fluorescent substances are photolabile. Chromatograms were examined under ultraviolet light. Each fluorescent spot was circled with a light pencil mark and its Rf (Ratio-to-front) value recorded for both the solvents. Quantification was accomplished by circling and cutting out the spots, cutting out blanks of corresponding sizes and eluting each with 4 ml of NH_4OH (1 ml concentrated NH_4OH diluted to 150 ml), allowing 1 to 3 hours for the extraction of the fluorescent substances. The fluorescence of the solution was measured with an Aminco-Bowman Spectrophotofluorometer using 366 mμ and 440 mμ as primary and secondary wavelengths and with slit arrangement 4 (GOLDSCHMIDT 1954).

The main pteridines, as observed in the wild type by HADORN (1959), are (1) drosopterins—the red eye-pigments having an orange fluorescence (three kinds of drosopterins have been found, drosopterin, isodrosopterin, and neodrosopterin); (2) isoxanthopterin—a deep violet blue fluorescent compound; (3) HB_1—2-amino-4-hydroxypteridine, a sky blue fluorescent compound; (4) HB_2—biopterin, a highly light-sensitive compound; (5) sepiapterin—a yellow pigment having a yellow fluorescence; (6) xanthopterin—a greenish blue fluorescent compound; (7) isosepiapterin—a yellow fluorescent compound.

RESULTS

The chromatograms for *cl* flies (Table 1) show a characteristic pattern with ten fluorescent substances, three in addition to those found in the *pn* mutants (Table 2), and all present in high concentrations. Although pn^{59j} arose in a clot stock, and is almost indistinguishable from *cl* under the microscope, it is biochemically distinct; only one substance is at the same concentration in both mutants.

Just as clot and prune can be distinguished, so *pn/pn*, pn^{59j}/pn^{59j}, and pn/pn^{59j} can be differentiated with experience. The eye color of freshly hatched *pn* flies is transparent brownish red, deepening with age to dark brownish purple. The pn^{59j} eye color, when freshly hatched, is more brown, less orange than *pn*; it also darkens with age.

Examination of the chromatograms (Table 2) show quantitative differences

TABLE 1

Quantitative analysis of the fluorescent substances in the eyes of clot, and their mean Rf values in solvents propanol ammonia and Collidine using ten samples each of 15 female flies

Fluorescent spots	Color of fluorescence	Intensity in clot	Mean Rf value in propanol ammonia	Mean Rf value in Collidine
1	orange (drosopterin-1)	.90 ± .053	.06	.07
2	light orange (drosopterin-2)	.72 ± .076	. .	appear as streak
3	greenish blue	.55 ± .033	.12	.25
4	violet blue (isoxanthopterin)	1.43 ± .054	.14	.30
5	greenish blue (xanthopterin)	3.46 ± .189	.22	.45
6	violet blue	2.18 ± .256	.20	.65
7	bright blue (kynurenine)	2.75 ± .144	.44	.13
8	blue (HB-1)	10.94 ± .267	.40	.48
9	blue (HB-2)	9.93 ± .329	.41	.51
10	yellow (sepiapterin)	15.28 ± .543	.43	.65

TABLE 2

Quantitative analysis of the fluorescent substances in the eyes of y pn, pn^{59j}, *and the heterozygote* y pn/+ pn^{59j} *flies using ten samples each of 15 female flies*

	Fluorescent substances	Intensity in *y pn*	Intensity in pn^{59j}	Intensity in pn/pn^{59j}
1	drosopterin-1	.21 ± .001	.18 ± .014	.38 ± .035
2	drosopterin-2	.33 ± .037	.30 ± .037	.29 ± .035
5	xanthopterin	1.09 ± .100	1.40 ± .158	1.15 ± .109
7	kynurenine	1.85 ± .166	2.88 ± .180	1.02 ± .076
8, 9	HB-pteridines	4.04 ± .106	6.16 ± .192	6.08 ± .195
10	sepiapterin	1.16 ± .172	2.10 ± .119	1.59 ± .139

between *pn/pn*, pn^{59j}/pn^{59j}, and pn/pn^{59j}. The same six fluorescent spots were produced but in different concentrations. By matching with the colors of the fluorescent spots and Rf values described by HADORN (1959), the pteridines are assumed to be drosopterins, xanthopterin, HB-pteridines, and sepiapterin. Chemical identification of the pteridines was not performed, and they have been numbered by the order in which they appeared in the chromatograms. Fluorescent Spot 7, which appears as a bright blue, is assumed to be kynurenine, from the description given by HANDSCHIN (1961). Kynurenine is a tryptophan derivative and a precursor of the brown eye-pigments (ommochromes).

There is a significant difference between *pn/pn*, and pn^{59j}/pn^{59j} females in the concentration of three fluorescent spots, kynurenine, HB-pteridines and sepiapterin (Table 2). The heterozygous females pn/pn^{59j}, have four spots, drosopterin, xanthopterin, kynurenine, and sepiapterin in the same concentration as the *pn* parent, and HB-pteridines the same as the pn^{59j} parent; they have twice the amount of drosopterin that is found in either parent.

The F_1 females from reciprocal crosses of *y pn* and pn^{59j} have darker eyes than either parent, but the eyes of F_1 males are also darker; therefore, allelic interaction is not an adequate explanation. The F_1 *y pn* males and F_1 pn^{59j} males obtained from reciprocal crosses of *y pn* and pn^{59j} should have the eye color of the female parent. Instead their eyes are darker than either parental stock.

TABLE 3

Quantitative analysis of the fluorescent substances in the eyes of pure strain and F_1 *males from reciprocal crosses between* y pn *and* pn^{59j}

	Fluorescent substances	Intensity of fluorescence			
		y pn stock	*y pn* F_1	pn^{59j} stock	$pn^{59j}F_1$
1	drosopterin-1	.32 ± .031	.53 ± .072	.22 ± .022	.40 ± .056
2	drosopterin-2	.16 ± .137	.42 ± .087	.19 ± .050	.66 ± .096
4	isoxanthopterin	.46 ± .193	.91 ± .178	.54 ± .071	.77 ± .085
5	xanthopterin	.98 ± .162	1.12 ± .122	1.31 ± .115	1.23 ± .148
7	kynurenine	1.11 ± .265	1.41 ± .188	2.82 ± .214	1.17 ± .078
8, 9	HB-pteridines	3.26 ± .433	3.16 ± .484	3.67 ± .374	3.86 ± .290
10	sepiapterin	.96 ± .117	1.05 ± .115	1.49 ± .126	1.20 ± .093

Two-dimensional chromatograms of the heads of *y pn* males and pn^{59j} males from the parental stocks and F_1 males obtained from the reciprocal crosses of the two stocks, were developed and subjected to a quantitative analysis (Table 3). The *y pn* males and pn^{59j} males of the stocks showed significant differences in three fluorescent substances (xanthopterin, kynurenine, and sepiapterin), but no significant difference in HB-pteridines which are found in small quantity in males. This is in contrast to the condition observed in females. The F_1 males always had greater quantities of drosopterins than the males of either parental line, accounting for the darker eye color. Kynurenine appeared in greater amounts in pn^{59j} males than in pn^{59j} F_1 males. Isoxanthopterin appeared in greater quantities in the *y pn* F_1 males than in *y pn* P_1 males. These differences between P_1 males and the F_1 males may be attributed to the interaction of the two backgrounds.

The chromatograms of the two different types of F_1 males, *y pn*/Y, and pn^{59j}/Y were also different and this may be attributed to the functional difference of the alleles, the pleiotropic effects of yellow, or other effects of the X chromosomes from different sources (for both kinds of males the autosomes are of common origin). The eye colors of the F_2 progeny showed various intensities of brown, ranging from dark brown to light orange-brown, indicating autosomal interaction.

To analyze the effect of *y* on the pteridine content of the eyes, chromatograms of the heads of wild-type males and heads of yellow males with wild-type eye color were developed (Table 4). No difference was observed between the two genotypes in their chromatographic patterns. Hence, *y* seems not to have measurable influence on the pteridine content of the eyes. The difference between the two stocks may be due to differences between the two alleles, differences in background, or both.

Since the F_1 *y pn* males and pn^{59j} males obtained from reciprocal crossing of the two stocks have the same autosomes and *y* seems unlikely to have any appreciable effect on the pteridine content of the eyes, any difference observed between them should for the most part be due to the difference in the two genes.

TABLE 4

Quantitative analysis of the fluorescent substances in wild-type eyes from a wild-type stock and from a yellow body stock (six samples of each genotype, 15 male flies per sample)

	Fluorescent substances	Intensity of fluorescence Wild-type males	Yellow-bodied males
1	drosopterin-1	.98 ± .066	1.14 ± .119
2	drosopterin-2	.48 ± .033	.44 ± .082
4	isoxanthopterin	1.24 ± .253	1.19 ± .329
5	xanthopterin	1.25 ± .257	1.18 ± .328
6	unknown	.41 ± .116	.54 ± .083
7	kynurenine	1.00 ± .178	1.16 ± .178
8, 9	HB-pteridines	4.16 ± .670	4.04 ± .280
10	sepiapterin	.85 ± .124	.68 ± .097

The difference between the two F_1 males is much less than the difference between the parental stocks. F_1 *y pn* males produced more HB-pteridines. In either case the difference was not significant. Known sources of nongenetic variations, including differences in age and in size of the flies, were controlled by using 3-day old adults and by replicating experiments.

DISCUSSION

Two mutants, pn^{59j} that arose in a *cl* stock, and *pn* obtained for allelism tests, are alleles in the classic sense. They produce the mutant phenotype in the heterozygous condition. On the other hand, recombinant types occurred in low frequency (.013 percent) and chromatographic patterns showed partial complementation. The amount of red eye-pigments (drosopterins) in *pn* and pn^{59j} is less than that of wild-type flies but the *trans* heterozygote pn/pn^{59j} produces more drosopterins than either parent, approaching the wild type in this respect.

The fact that F_1 females have darker eyes than either parent could be interpreted as allelic interaction. The F_1 males should have eyes of the same shade of prune as the female parent if inheritance follows the classic sex-linked pattern. However, the eye color of F_1 males is darker than either parent strain and is indistinguishable from the eye of the F_1 females. Chromatographic patterns produced by F_1 males obtained from reciprocal crosses differed considerably from patterns produced by their "uncles." This must be interpreted as due to the influence of autosomes, as the X chromosomes of F_1 males and males of the parental strain are alike.

In addition to allelic difference, the difference in the concentration of pteridines between the eyes of *pn* and pn^{59j} could be due to the different sets of autosomes they possess, and also to the presence of *y* in one type and not in the other. As *y* has been shown to have no measurable effect on the pteridine content of the eyes, the effects of *y* may be ignored. The effects of the autosomes cannot be disregarded. The phenotypic expression of a mutant gene may be altered by its background so, unless there is rigid control of the background, any suggestion of synergistic effects of allelic substitutions must be viewed with caution.

The chromatographic patterns of male and female flies of *pn*, pn^{59j} or F_1 are different. Both sexes produce the same amount of drosopterins and the color of the eyes of male and female flies is indistinguishable. Isoxanthopterin occurs in the chromatograms of males but not in females. It has been observed (HADORN 1959) that wild-type adult males contain about 0.5 μg isoxanthopterin whereas females of the same age had only one fourth this amount.

The males have less HB-pteridines and more isoxanthopterin than the females. This suggests a relationship between HB-pteridines and isoxanthopterin. FORREST, GLASSMAN and MITCHELL (1956) have demonstrated the enzymatic oxidation of 2-amino-4-hydroxypteridine to isoxanthopterin and xanthine to uric acid. Also, HADORN (1959) demonstrated that $rosy^2$ flies, lacking xanthine dehydrogenase, accumulated 2-amino-4-hydroxypteridine in the testes and hypoxanthine was passed as an excretory product instead of uric acid. Presence of less

HB-pteridine and more isoxanthopterin in the males, in our study, could be due to more xanthine dehydrogenase in the males than in the females. However, MUNZ (1962) observed that females exhibited a higher enzyme activity than males though males had more isoxanthopterin than female imagos. That the heterochromatic Y is not responsible for differences between the sexes in pteridines is indicated by the demonstration by Fox (1954) that females with Y, Y^L, or Y^S show the typical female chromatographic pattern.

SUMMARY

The mutant allele of prune pn^{59j}, that arose in a clot eye stock, is visibly distinguishable from *cl* and *pn*; it also differs in chromatographic pattern (three pteridines found in *cl* are absent, two plus kynurenine are in different concentration than *pn*). Heterozygous females pn/pn^{59j} have four substances in the same concentration as the *pn* parent, one like pn^{59j}, and drosopterin-1 in twice the amount found in either parent (in this respect, approaching the wild type). There were two wild-type flies among 30,617 F_2 flies examined. Differences between F_1 males and P_1 females are attributed to the influence of genes in the autosomes.

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