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The inheritance of lymph proteins in *Drosophila*

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1. INTRODUCTION

Despite the impressive number of mutants known in *Drosophila melanogaster*, very few of the mutations are detectable by differences in individual proteins. We describe here the mode of inheritance of three protein fractions each controlled by a different gene.

2. MATERIALS AND METHODS

(i) Drosophila *stocks*

A number of standard laboratory mutant stocks were obtained by courtesy of Dr Charlotte Auerbach of the Institute of Animal Genetics, Edinburgh. Also, cultures were set up from flies caught in the Belfast area.

(ii) *Electrophoresis*

Lymph was obtained by tearing open third instar larvae and absorbing the fluid emerging onto fine strips of filter paper which were then used as sample holders for electrophoresis. The starch plates were prepared and the whole procedure of starch electrophoresis was carried out in the now standard method of Smithies (1955), using the discontinuous buffer system of Poulik (1957). The variables were as follows: 5 V./cm. length, 1 mA./cm. width, length of run, about 15 hr.; distance covered by the 'front' about 12 cm. The starch plates were stained in Amido Black 10 B.

3. EXPERIMENTS AND RESULTS

In exploratory experiments, each sample consisted of pooled lymph from 3–4 third instar larvae, and 22 different stocks were tested. Up to seven distinct bands of varying intensity appeared, justifying the expectation that separation of fractions would be possible. The patterns obtained suggested also that there were phenotypic differences between and, possibly, also within stocks.

It was decided therefore to repeat the survey of stocks using a single larva per sample, and it was thus confirmed that several phenotypes may appear within one stock. As an illustration, some of the patterns obtained are shown in Plate 1. We selected three fractions or bands (marked A, B and C in the illustration) as the subject of our first investigation.

In order to test whether and in what way these three fractions are inherited, it would be desirable of course to set up pair-matings of known phenotypes. In practice this proved impossible, because one needs a sample of lymph to identify the phenotype, but once the lymph is withdrawn the larva dies, and no offspring from it can be obtained. To by-pass this difficulty we set up many pair-matings from within our Bar-eye stock, and tested samples from the F_1 and in some cases from the F_2; hoping in this way to draw inference as to the phenotypes (and genotypes) of the parents, and to obtain possible homozygote lines. We were also helped by the fact that individual sampling from a culture of the *brown-eye* stock showed this to be uniform, exhibiting constantly the first two fractions, but lacking the third.

The results of testing electrophoretically the offspring of a number of pair-matings will now be considered taking each fraction separately.

(iii) *First fraction*

The results of seven pair-matings are set out in Table 1. It will be noted that in matings 1, 2, 3, 5 and 7 *all* F_1 females but only *some* males have this fraction.

Table 1. *F_1 phenotypes from seven pair-matings within the* Bar eye *stock*

	Observed phenotypes											
	1 – 2 – 3 –		1 – 3 –		2 – 3 –		3 –		1 – 2 –		2 –	
Mating	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1	2	5	6	7	0	3	0	1				
2	3	2	6	1	0	3	0	5				
3	10	10			0	7						
4	7	4	1	4					4	2		
5			6	11			0	4				
6	3	2			1	1						
7	1	6			0	2			2	3	0	1

This suggests sex-linkage. In mating 6, both cases are represented in both sexes, and in mating 4 all individuals tested (of both sexes) show the fraction. These results are all in agreement with the hypothesis that presence of the fraction is governed by a sex-linked dominant gene, and absence by its recessive allele. The genotypes of the parents would be as follows:

Matings 1, 2, 3, 5, and 7: Female Aa, Male A
Mating 6: ,, Aa, ,, a
,, 4: ,, AA, ,, A, or a

Confirmatory matings were set up, using females from the F_1 of mating 6 and males from the F_1 of mating 3. The results are in agreement with the above conclusions; this is illustrated by mating 8 (Table 2). The P_1 genotypes in this mating would be: female *aa*, male AY.

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Table 2. *F_1 and F_2 phenotypes from mating* 8*—first fraction*

	Fraction present		Fraction absent	
	♀♀	♂♂	♀♀	♂♂
F_1	10	0	0	10
F_2	10	4	9	10
	14		19	
Expected	16·5		16·5	
χ^2	0·76 ($P_{5\%} = 3{\cdot}84$)			

(iv) *Second fraction*

In the F_1 of matings 1 and 2, some larvae lack the second fraction whilst in matings 3 and 7 all larvae tested show it; in all cases, irrespective of sex. It may be concluded from this that a gene governing the fraction would be autosomal and not sex-linked.

The question whether presence of the fraction is due to the dominant or to the recessive allele may be answered from a consideration of two matings, 9 and 10 (Table 3). Pair-mating 9 was between a female of our *bw* stock (all larvae have the second fraction), and a male from the F_1 of pair-mating 5 (all males lack this fraction). Mating 10 was reciprocal to 9.

Table 3. *Parental, F_1 and F_2 phenotypes from pair matings* 9 *and* 10. *Second and third fractions.*

Generation	Mating 9	Mating 10
Parental	♀ 2− × ♂ 3−	♀ × ♂ 2− 3−
F_1	All 2− 3−	All 2− 3−
F_2: types	2− 2− 3− 3−	2− 2− 3− 3−
observed	6 14 6	19 30 8
expected	6·5 13 6·5	14·25 28·5 14·25
χ^2 (2 d.f.) $P_{5\%} = 5{\cdot}99$	0·16	4·38
Deduced parental genotypes	$\frac{B}{c}\frac{B}{c}$ × $\frac{b}{C}\frac{b}{C}$	$\frac{b}{C}\frac{b}{C}$ × $\frac{B}{c}\frac{B}{c}$

(v) *Third fraction*

As in the case of the second fraction, the results listed in Table 1 suggest an autosomal gene for the third fraction also. The *bw* stock proved useful again; it constantly shows the second but lacks the third fraction. This indicates that the

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latter is governed by a distinct gene. This stock is presumably homozygote for the third fraction, and has provided the female of mating 9 and the male of mating 10. The other parent was obtained in each case from a particular culture vial of *Bar eye* stock, in which all larvae lacked the second but showed the third fraction.

The results, set out in Table 3, lead again to the conclusion of an autosomal dominant for presence and a recessive for absence of the protein band concerned.

Furthermore, it will be noted that the F_2 generation of these dihybrid crosses (i.e. matings 9 and 10) comprise only three phenotypes instead of the four expected on the assumption of independent assortment; suggesting linkage between the genes for the second and third fractions. The description of the phenotypes is in agreement with the conclusion of linkage, and the numbers for each phenotype in the small sample tested also agree with the expected ratios (see Table 3).

4. DISCUSSION

The now classical studies of Ephrussi, Beadle and others demonstrated that certain alternative eye colour phenotypes in *Drosophila*, namely vermilion (*v*) and cinnabar (*cn*), are due to the interruption, at various points, of the chain of reactions leading to the formation of the brown pigment. In the case of vermilion the blockage was located in the step: tryptophane → formyl kynurenine, and has been attributed to absence of the corresponding enzyme, tryptophane peroxidase, an assumption which was confirmed later (Baglioni, 1959).

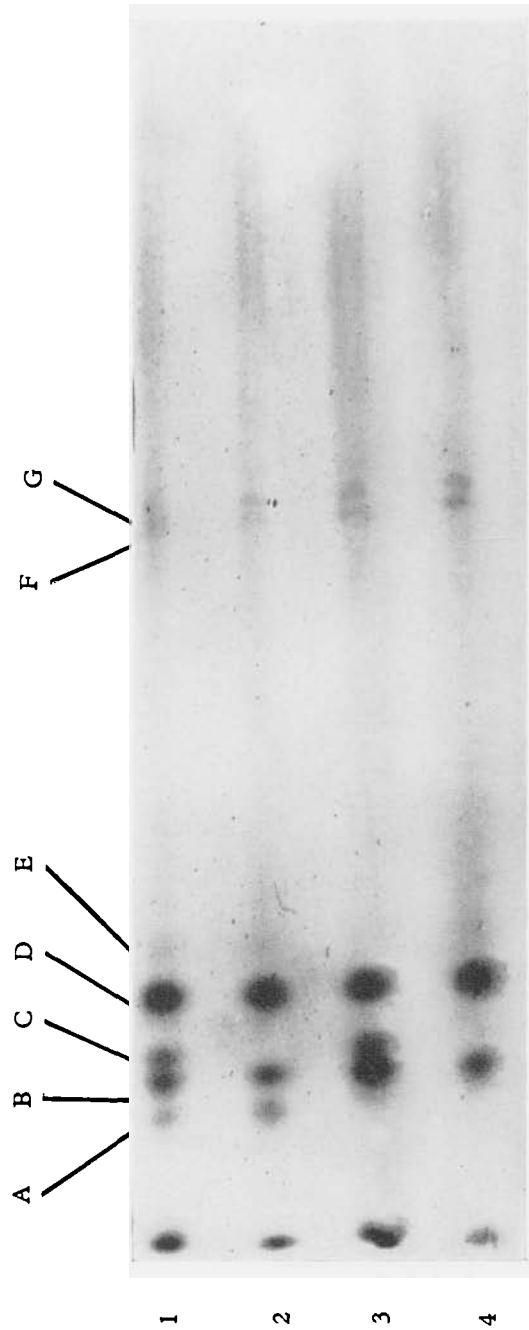
Similarly, in the reaction series leading to the formation of the red eye pigment, the mutant, *rosy* (*ry*) and others fail at the step: 2-amino-4-hydroxypterin→iso-xanthopterin, due to the absence of the enzyme, *xanthine dehydrogenase*. (Reviews for both cases: Ephrussi, 1942; Glass, 1957; Ziegler, 1961; Chovnik, *et al.* 1962).

These are probably the first cases in *Drosophila* where the primary action of the gene at the level of the synthesis of a protein (the enzyme) has been demonstrated. Relevant also is the study of Chen (1956), who compared by paper electrophoresis the lymph proteins of normal and lethal-translucida (*l-tr*) larvae. The aberrant genotype results in this case in a rather general failure of protein synthesis. Whilst the lethal larvae show a great reduction in lymph peptide and protein content, they contain large amounts of aminoacids.

In the *Cecropia* silkworm, a protein restricted to the female and eventually passing into the eggs has been detected immunologically by Telfer (1954); but the genetics of this sex-limited character has not been investigated.

It follows that the present case is one of the very few in *Drosophila* where the presence or absence of a protein fraction, under the control of a single gene, is directly detected.

The sex-linkage shown for the first fraction is of interest in view of the fact that human congenital a-gamma-globulinaemia is also caused by a sex-linked mutant. Again in agreement with the human mutant as well as with the eye-colour mutants is the fact that for all three fractions studied, the dominant allele ensures presence of the protein whilst absence is due to the recessive.



Photograph of parts of an electrophoretic starch plate. Examples of phenotypes obtained from single-larva lymph samples in the *Bar eye* stock. A–G, fractions separated. A, B, C, the three fractions with which this paper is concerned. 1. Phenotype with A, B and C present. 2. A and B present. 3. B and C present. 4. B present.

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Possible effects of the absence of a fraction on viability and development, as well as the mode of inheritance of fractions other than the three so far studied, are the subjects of further investigations now in progress. A comparison of the lymph protein pattern of successive developmental stages is reported in another paper (Duke & Pantelouris, 1964).

5. SUMMARY

The inheritance of three lymph protein fractions separable by starch-plate electrophoresis has been studied. Each fraction is under the control of a separate gene, the dominant allele determining its presence and the recessive its absence.

The gene for the fastest fraction is sex-linked, whilst those for the other two are autosomal and linked.

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