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Further studies on the inheritance of lymph proteins in *Drosophila melanogaster*

By E. J. DUKE

*Zoology Department, Queen's University, Belfast, Northern Ireland**

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1. INTRODUCTION

Pantelouris & Duke (1963), using starch gel electrophoresis have detected nine to eleven distinct protein fractions in the late third instar larval lymph of the Bar eye (*B*) mutant stock of *Drosphila melanogaster*. They also described the mode of inheritance of three of these protein bands; each is controlled by a different pair of alleles. In the present paper, the mode of inheritance of three of the remaining protein fractions is reported.

The possibility that a fraction on starch gel might be resolved into two or more by other methods of electrophoresis cannot be excluded. A comparison was, therefore, made by two-dimensional electrophoresis of the larval lymph protein pattern on either polyacrylamide gel or cellulose acetate paper in the first stage, and starch gel in the second.

2. MATERIALS AND METHODS

Drosophila *stocks*

Starch gel electrophoresis has shown (Fig. 1) that the brown eye (*bw*) mutant stock of *Drosophila melanogaster* is uniform for ten lymph protein fractions (Duke & Pantelouris, 1963). This stock is, therefore, very useful in breeding experiments for the investigation of the inheritance of the different proteins.

A stock called Pacific 7 was obtained through the courtesy of Dr Forbes Robertson of the Institute of Animal Genetics, Edinburgh. This proved to be a most useful stock, as individual sampling indicated the complete absence of protein bands 8 and 13 (see Fig. 1).

A series of cultures were started from flies caught in various areas of Ireland. One of these, named the Belfast II stock, was found to lack protein fraction 9 (see Fig. 1).

Electrophoresis

Starch gel electrophoresis of *Drosophila* lymph was carried out by the method of Smithies (1955), with the discontinuous buffer system (pH 8·6) of Poulik (1957).

* Present Address: Department of Biochemistry, School of Medicine, University of North Carolina, Chapel Hill, North Carolina.

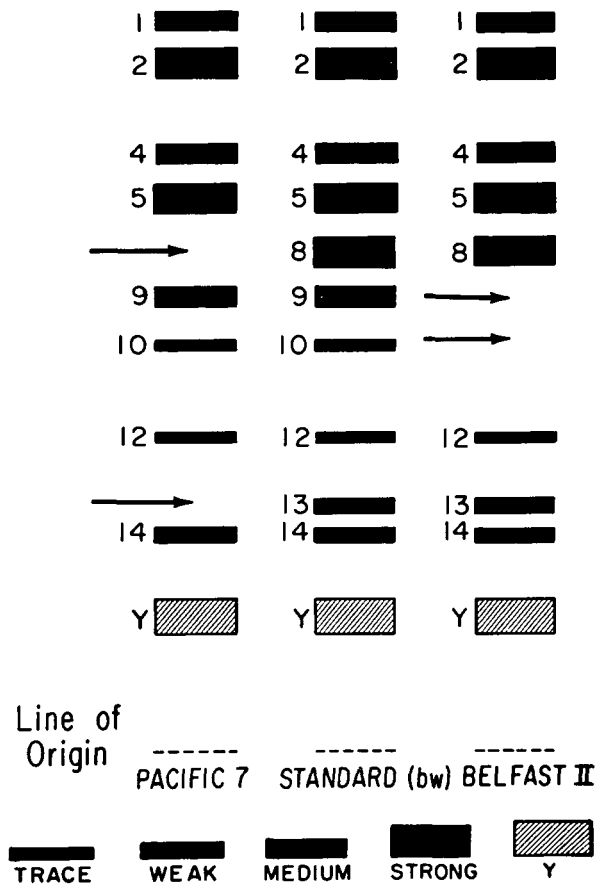

Fig. 1. Diagrammatic representation of lymph protein differences between three stocks of *Drosophila melanogaster*. The arrows indicate where the stocks differ from one another.

Lymph for electrophoresis was obtained as described by Duke & Pantelouris (1963). A constant current of 1 mA/cm. length was maintained across the starch plate until the 'front' line had migrated 12 cm. from the origin. Staining was made with a saturated solution of Amido Black 10 B in 50 parts methanol:50 parts water:10 parts acetic acid.

The cellulose acetate method of Kohn (1957*a*,*b*) with the buffer modification of Aronsson & Gronwall (1957) was employed. The pH of this system was 8·9. The lymph was applied by perforating the larval abdomen with a tungsten needle and allowing it to soak in along the line of origin. A voltage gradient of 6 V./cm. was applied across the strip and a running time of 15 hours allowed. The ribbon was fixed in heat at 110°C. and stained in Lisamine Green.

Polyacrylamide electrophoresis was carried out as described by Ornstein & Davis (1962) The buffer pH values of the electrode chamber and the small pore gel were 8·4 and 8·9 respectively. Application of the lymph sample was made in a layer of 50% sucrose. The best results were obtained by perforating the abdomen of six-ten

late third instar larvae and allowing the lymph to drain into 0·15 ml. of 50% sucrose. A constant current of 5 mA. per tube was employed and the gels were stained in Amido Schwarz.

Two-dimensional electrophoresis between starch gel and cellulose acetate was carried out in similar fashion to that described by Duke (1963). In the case of the polyacrylamide–starch gel comparison, the sample was first run on the former medium and then the entire gel was placed in a slot in the starch, at right angles to the direction of current, in such a manner that close contact between the two gels was made. A control pattern in the same starch gel enabled two-dimensional comparisons to be made.

3. EXPERIMENTS AND RESULTS

Figure 1 shows the late third instar larval lymph protein patterns of the three stocks used in the present study. The numbering of fractions is as previously used (Duke & Pantelouris, 1963). The mode of inheritance of fractions 8, 9 and 13 is studied here. We shall now consider each fraction separately.

Fraction 8

The brown eye (*bw*) stock, which constantly exhibits fraction 8, was crossed reciprocally with the Pacific 7 stock, which uniformly lacks the fraction. Table 1 shows the results of these crosses.

Table 1. *Fraction* 8. *Parental,* F_1 *and* F_2 *phenotypes from the reciprocal crosses* bw (*fraction present*) × *Pacific* 7 (*fraction absent*)

Mating*	♀(+) × ♂(−)		♀(−) × ♂(+)		
	♀♀	♂♂	♀♀	♂♂	
F_1 +	12	10	11	13	
−	0	0	0	0	
	♀♀	♂♂	♀♀	♂♂	Total
F_2 +	25	23	28	24	100
−	8	5	6	7	26

$\chi^2 = 1{\cdot}28$ for deviation from 3:1
$P\,(5\%) = 3{\cdot}84$.

* +, − indicate presence and absence respectively of fraction 8.

The F_1 generation results showed that the gene, or genes, controlling presence of fraction 8 was dominant in character as all F_1 heterozygotes exhibited the fraction very strongly.

In the F_2 generation segregation occurred independently of sex into ratios approximating the typical Mendelian 3:1 ratio of a monohybrid cross. The observed values, as seen in Table 1, statistically fit the hypothesis of a single autosomal gene segregating in such a fashion.

In summary, therefore, fraction 8 is governed by a single autosomal gene, with the dominant allele responsible for presence and the recessive for absence.

Fraction 9

For the study of this fraction reciprocal crosses were set up between the *bw* stock, which contains fraction 9, and the Belfast II stock which lacks it (Fig. 1).

In the F_1 generation this fraction, which is normally quite faint, was much more difficult to detect. In males and females of both crosses it only appeared when the pooled samples were made quite concentrated. In cases where only one larva was used the fraction could not be detected. The technique, therefore, did not distinguish the heterozygote from the homozygote which completely lacks the protein. The F_2 individuals were investigated with this in mind.

Results obtained in the F_2 generations are shown in Table 2. Segregation again occurred in such a manner that a single gene system seemed to be in operation.

Table 2. *Fraction* 9. *F_2 phenotypes from the reciprocal crosses*, bw (*fraction present*) × *Belfast II* (*fraction absent*)

Mating*	♀(+) × ♂(−)		♀(−) × ♂(+)		
	♀♀	♂♂	♀♀	♂♂	Total
F_2 +	6	6	6	4	22
−	16	15	18	11	60

χ^2 = 0·15 for deviation from 3:1
P (5%) = 3·84

* +, − indicate presence and absence respectively of fraction 9.

The fraction was expressed independently of sex, indicating autosomal inheritance. The ratio of individuals lacking fraction 9 to those exhibiting it was very close in all cases to a 3:1 ratio. The evidence obtained from the F_1 generation indicated that the heterozygote, although containing some fraction 9, would be scored similarly to the homozygote which completely lacks it. The results fit the hypothesis, therefore, that expression of fraction 9 is governed by a single pair of semi-dominant autosomal alleles.

Fraction 13

The same reciprocal crosses which were used in the investigation of the inheritance of fraction 8, could also be used to study the inheritance of fraction 13 and, in addition, to detect whether the two gene systems are linked. The *bw* stock constantly exhibits fractions 8 and 13 while the Pacific 7 stock constantly lacks them (Fig. 1).

In the F_1 generation the fraction appeared in all individuals of both crosses, showing that presence of the protein is dominant.

Fraction 13 appeared independently of sex in F_2 individuals so that no sex-linkage is involved. The individuals exhibiting the protein fraction outnumbered those lacking it by ratios which statistically fit the 3:1 ratio expected from a single gene

Table 3. *Fraction* 13. *F_2 generation phenotypes from the reciprocal crosses,* bw (*fraction present*) × *Pacific* 7 (*fraction absent*)

Mating*	♀(+) × ♂(−)		♀(−) × ♂(+)		
	♀♀	♂♂	♀♀	♂♂	Total
F_2 +	17	17	14	12	60
−	7	6	6	5	24

$\chi^2 = 0{\cdot}57$ for deviation from 3:1
$P(5\%) = 3{\cdot}84$

* +, − indicate presence and absence respectively of fraction 9.

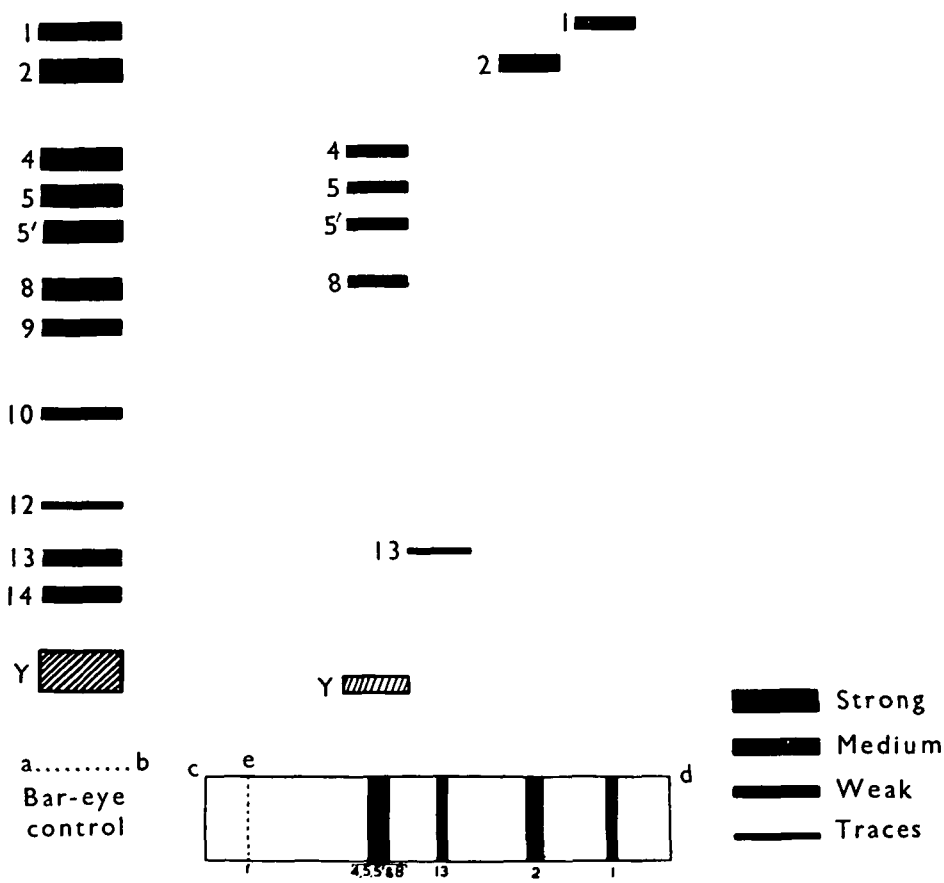

Fig. 2. Diagrammatic representation of two-dimensional electrophoresis of larval lymph from the Bar eye stock of *Drosophila melanogaster*. The first stage was on cellulose acetate and the second on starch gel. ab = Origin of the control Bar eye lymph pattern; cd = origin of the acetate in starch gel; ef = origin of original Bar eye lymph on cellulose acetate.

system behaving in normal Mendelian fashion. The inheritance of this protein fraction resembles that of fraction 8 in that it is governed by a single autosomal gene where presence is determined by the dominant allele and absence by the recessive. No evidence of linkage between the above two genes was observed.

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Two-dimensional electrophoresis

The results of the two-dimensional electrophoresis of larval *Drosophila* lymph in cellulose acetate and starch gel, also in polyacrylamide and starch gels, are shown diagrammatically in Figs. 2 and 3 respectively.

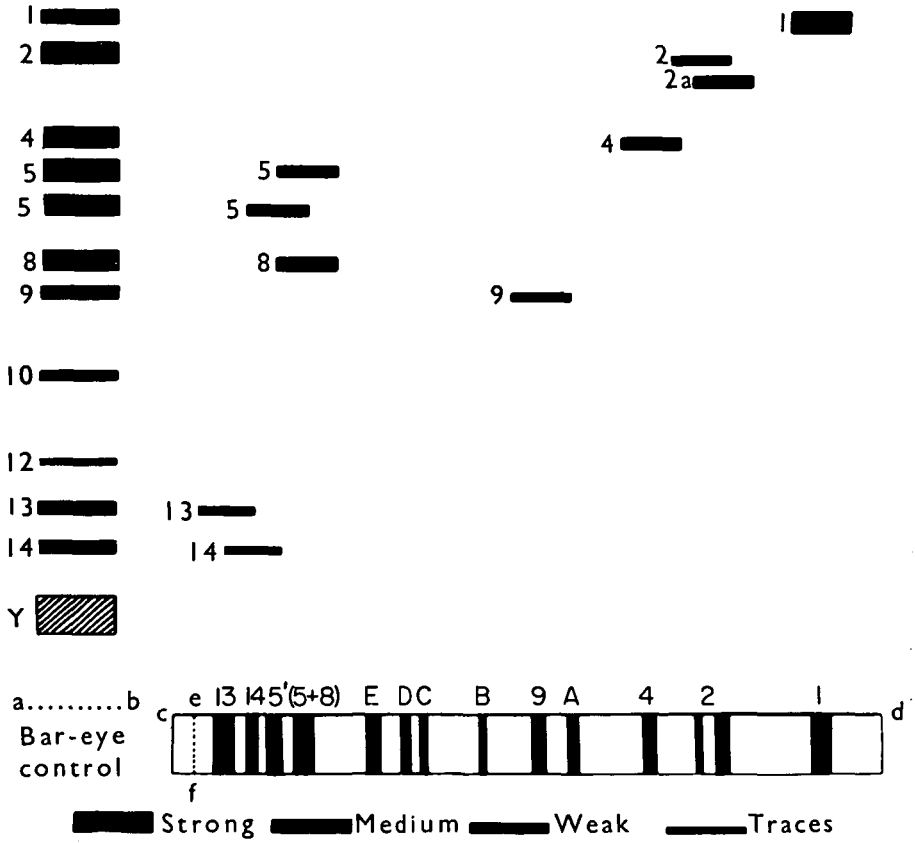

Fig. 3. Diagram of two-dimensional electrophoresis of larval lymph on polyacrylamide and starch gels. Polyacrylamide fractions A–E could not be traced in starch gel. ab = Origin of the control Bar eye lymph pattern in the starch gel; cd = origin of the polyacrylamide gel in the starch gel; ef = origin of the original Bar eye lymph sample in the polyacrylamide.

Cellulose acetate electrophoresis gave only four clear lymph protein fractions (Fig. 2). None of the starch gel protein fractions were found to be sub-divided by this alternate method. In fact, the slowest fraction on cellulose acetate was divided into fractions 4, 5, 5′, 8 and the diffuse area Y by the starch gel method. The faint fractions, 9, 10, 12 and 14 on starch gel, could not be traced on cellulose acetate.

Polyacrylamide gel electrophoresis detected up to fourteen protein discs in larval *Drosophila* lymph, which is three more than was obtained with starch gel (see Fig. 3). From the two-dimensional analysis it appeared that only starch gel fraction 2 was subdivided by the polyacrylamide technique. This fraction, the inheritance of

which has not yet been worked out, can also be sub-divided on starch gel when a high voltage in the region of 300 V. is employed. The five fractions A–E of the polyacrylamide pattern (see Fig. 3) could not be detected in the starch gel. Apart from fraction E these proteins were faintly expressed and so it is not surprising that they could not be detected in the larger starch gel medium. When a polyacrylamide gel was stained for protein after being used as the first stage in a two-dimensional experiment, a fraction corresponding in position to E was observed.

4. DISCUSSION

Many gene–enzyme systems have been identified in diverse organisms including *Drosophila melanogaster* (for bibliography, see Shaw, 1965). These systems were all detected on the basis of the proteins having enzymatic activity. Apart from the gene-controlled lymph protein systems described in this paper, only two others of a non-enzymatic nature have been observed in *Drosophila*. Hubby (1963), in a survey of adult soluble proteins using polyacrylamide electrophoresis, found only one major protein difference. A densely staining protein band in the glass^3 (gl^3/gl^3) stock was found to have a slightly different mobility from the most densely staining band in the Oregon R stock. Heterozygotes in reciprocal combinations revealed both bands in approximately half the concentrations found in the parental stocks. This effect bears a resemblance to the results described above for fraction 9. Pantelouris & Duke (1963) described the inheritance of protein fractions 4, 5, and 5′ within the Bar-eye (B) stock. The present paper represents a continuation of this latter work.

The inheritance of each larval lymph protein fraction so far described for *D. melanogaster* is under the control of a single structural gene. Furthermore, in all but two of these seven systems, presence of the protein is dominant and absence recessive. It is interesting to note also that the system governing fraction 4 is the only one so far described which exhibits sex-linkage. Work is now in progress to map in detail the genes governing the three protein fractions described here and also the three fractions described by Pantelouris & Duke (1963).

Two-dimensional electrophoresis did not show any definite sub-fractionation on cellulose acetate or on polyacrylamide gel of those starch gel protein fractions which are governed by single structural genes. Some faint polyacrylamide fractions could not be traced in the larger starch gel medium by the two-dimensional technique, so that such sub-fractionation is not completely ruled out.

SUMMARY

The inheritance of three protein fractions of third instar larval lymph in *Drosophila melanogaster* as detected by starch gel electrophoresis is described. Each of the three is governed by a single autosomal gene. In two, the dominant allele determines presence, and the recessive absence of the respective fraction. The third protein fraction is governed by a single pair of co-dominant alleles.

Comparison of the starch-gel pattern to that obtained on cellulose acetate and on polyacrylamide gel fails to show definite sub-fractionation of those protein bands of which the inheritance has been studied.

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