location is in agreement with the genetic map position of 6.5-7.0 determined above.

Acknowledgements: Thanks to Dr. Adelaide T. C. Carpenter for helpful criticism and advice; thanks also to SJB, IE, KP, and KC for stimulating and productive support.

References: Baker et al. 1978, Genetics 90:531; Baker, B.S. and A.T.C. Carpenter 1972, Genetics 71:255; Carpenter, A.T.C. and L. Sandler 1974, Genetics 76:453; Lindsley, D.L. and E.H. Grell 1968, Carnegie Inst. of Wash. Publ. No. 627.

Lee, T.J. Chungang University, Seoul, Korea. Sexual isolation among four species in the Drosophila auraria complex.

The D. auraria complex was divided into four species, D. auraria, D. biauraria, D. triauraria and D. quadraria (Bock and Wheeler 1972). The sexual isolation among three species, D. auraria, D. biauraria, and D. triauraria, was significantly demonstrated (Kurokawa 1960; Lee 1970).

For the experiment of mating preference a usual male multiple choice method was used. Results of the tests are summarized in Table 1. It is noted that the sexual isolation showed a weak degree in all of the crosses except for one case. A higher sexual isolation was seen in the crosses with D. quadraria males than in the reverse cases with D. auraria males. This difference caused by the males may be partly attributed to the morphological difference between their genitalia. It can hardly be concluded from morphological, physiological and distributional studies (Lee 1974a, 1974b) that, of the members belonging to species D. auraria complex, D. quadraria would be the ancestral species.

Table 1. Sexual preference tests among four species.

Crosses ♀♀	♂♂	Homogamic (%)	Heterogamic (%)	Isolation index	Coefficient of joint isolation
A , D X	A	45	51	0.063	0.338
A , D X	D	71	17	0.614	
B , D X	B	69	50	0.160	0.088
B , D X	D	64	62	0.016	
C , D X	C	50	63	0.115	0.162
C , D X	D	78	51	0.209	

(A: D. auraria, B: D. biauraria, C: D. triauraria, D: D. quadraria)

References: Kurokawa, H. 1960, Jap. J. Gen. 35:161-166; Lee, T.J. 1974a, Rev. Tech. & Sci., Chungang Univ. 1:9-16; Lee, T.J. 1974b, Theses Collection, Chungang Univ. 19:63-73.

Leigh Brown, A. J. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Molecular weights of seven Drosophila enzymes.

Recent work in our laboratory (Leigh-Brown and Langley 1979; Leigh-Brown and Voelker 1979) has involved the estimation of the native molecular weight of several Drosophila enzymes for which such data were not previously available (O'Brien and MacIntyre 1978). As our earlier report gave only the results, I present here the methods used and the data on which those estimates were made.

Determination of sedimentation constants ($s_{20,w}$) by sucrose density gradient sedimentation was carried out according to the procedure of Martin and Ames (1961). Gradients were made in 5 ml cellulose nitrate tubes by layering 1.15 ml of each of 20%, 15%, 10% and 5% solutions of sucrose in 0.05M Tris-HCL pH 7.5 with 1 mM dithiothreitol (Sigma). They were then stored at 4°C for 24 hours. Crude fly homogenate was prepared in the same buffer by homogenising 0.5 g cn bw; ri e flies, centrifuging in the homogenate for 20 minutes at 15,000 rpm, and filtering through glass wool. The extract was then passed through a 40%/80% ammonium sulphate precipitation step and was diluted until the protein concentration, measured by $O.D._{260}/O.D._{280}$, was less than 20 mg/ml. Rabbit muscle Ldh was added (800 units/ml) and 0.1 ml was layered on top of each gradient. Three such gradients were centrifuged for 15.5 hr at 39,000 rpm in a Beckman SW 51 rotor at 4°C. After the run, two-drop fractions were collected on ice and assayed. Rabbit muscle lactate dehydrogenase and D. melanogaster alcohol dehydrogenase were used as standards.

Gel filtration was performed according to Andrews (1965). 5 g of frozen flies of the same strain were homogenized in cold 0.1M Potassium phosphate buffer, containing 1mM DTT and 1mM EDTA. The extract was spun, filtered and passed through a 40%/80% ammonium sulphate cut as before. After dialysis against 1 liter of buffer for 24 hours it was concentrated by ultrafiltration. One ml was applied to a column of "Sephacryl" S-200 (Pharmacia) of dimensions 88.0 x 2.0 cm. A flow rate of 1 ml/minute was maintained with a peristaltic pump and 20 drop fractions were collected. The column was first calibrated with α-chymotrypsinogen (M.W. 25,000), ovalbumin (45,000) and rabbit muscle aldolase (158,000), all assayed by measuring O.D.280. D. melanogaster Adh and aldolase were used as internal standards during the run. All operations were performed at 4°C.

Table 1.

		Sucrose density gradients $s_{20,w}$	Gel filtration $(-\ln K_{av})^{1/2}$ (Laurent & Killander, 1964)	Stokes radius Å	Molecular weight ($\times 10^{-3}$D)
Pgk	Mean	3.5	1.0343	27.3	39
	Se	0.11			
Tpi	Mean	4.0	1.0672	28.6	47
	Se	0.13			
Gpt	Mean	5.9	1.2526	35.9	87
	Se	0.19			
Pgi	Mean	6.6	1.2440	35.5	97
	Se	0.23			
Adh		3.9			
Ldh		7.45			
Chymotrypsinogen			0.8764	22.6	
Ovalbumin			1.0693	27.6	
Aldolase			1.4796	47.4	

In Table 1 is given the mean sedimentation constant ($s_{20,w}$) obtained from sucrose density gradients for Phosphoglycerate kinase (Pgk), Triose phosphate isomerase (Tpi), Glutamate pyruvate transaminase (Gpt) and Phospho-glucose isomerase (Pgi), with the standard errors from six estimations against the standards Adh and Ldh. The Stokes radii of these four enzymes were estimated from "Sephacryl" S-200 gel filtration after the method of Laurent and Killander (1964). These are also given in Table 1, with the elution data on the standards. From these data the molecular weights were calculated via the relationship:

$$M = \frac{6\pi\eta\, Nas}{1 - \bar{v}\,\rho}$$

where N = Avogadro's number (6.022×10^{23}); $\bar{v}$ = the partial specific volume (assumed to be 0.725 cm^3g^{-1} for globular proteins); η and ρ correspond to the viscosity and density respectively of the medium; and a = Stokes radius. The following values were obtained: Pgk, 39,000D (monomer); Tpi, 47,000D (dimer);

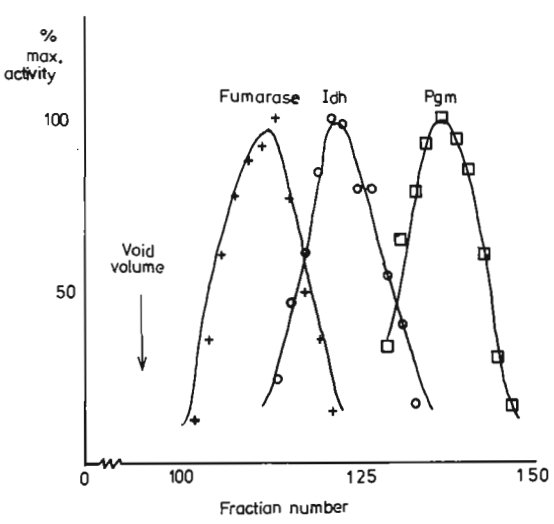

Fig. 1a

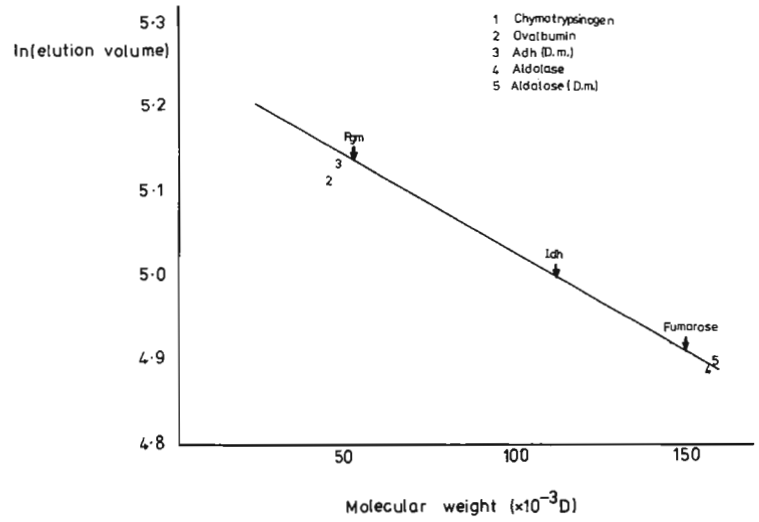

Fig. 1b

Gpt, 87,000D (prob. dimer); Pgi, 97,000D (dimer).

The molecular weights of three enzymes were estimated directly from the gel filtration data by the method of Andrews (1965). Their elution profiles and position relative to the standards are shown in Figure 1a and 1b. Estimates of 51,000, 112,000 and 150,000 were obtained from Pgm (monomer), Idh (dimer), and fumarase (tetramer), respectively, using the least-squares regression from the five standards: $y = 5.257-0.002319x$. Fractions from this column were also assayed for activity of dipeptidase isozymes by starch gel electrophoresis. By this means it was possible to assign approximate molecular weights of 120,000 and 170,000 Daltons to the Dip-A and Dip-B isozymes, respectively (Voelker, Ohnishi and Langley 1979).

References: Andrews, P. 1965, Biochem. J. 96:596-606; Laurent, T.C. and J. Killander 1964, J. Chromat. 14:317-330; Leigh Brown, A.J. and C.H. Langley 1979, Nature (in press); Leigh Brown, A.J. and R.A. Voelker 1979, Biochem. Genet. (in press); Martin, R.G. and B.N. Ames 1961, J. Biol. Chem. 236:1372-1379; O'Brien, S.J. and R.J. MacIntyre 1978, In Genetics and Biology of Drosophila, Ashburner, M. and T.R.F. Wright (Eds.), Vol. 2a, pp. 396-551, Academic Press, London; Voelker, R.A., S. Ohnishi and C.H. Langley 1979, Biochem. Genet. (in press).

Lindsley, D.E., L.S.B. Goldstein and L. Sandler. University of Washington, Seattle, Washington. Male sterility in maternal-effect mutants.

Seven mutations have been isolated in region 30-31 of chromosome 2 -- the so-called da-abo region. Of the five mutants that have been analyzed, all cause a maternal effect resulting in sex-specific embryonic lethality. In all cases, the severity of the maternal effect is sensitive to the heterochromatic constitution of the zygote, and the severity of the maternal effect is reduced if the experiments are carried out at 19° instead of 25°. The proposal was made that these five mutations define a cluster of functionally related genes (Sandler 1977).

We here report evidence that another of the seven mutations, mfs48, is also a member of the da-abo cluster. This evidence takes two forms: (1) mfs48 maps within the cluster, and (2) mfs48 exhibits phenotypic similarities to hup, one of the five known mutants in the cluster. On the basis of phenotype data presented here, we suggest a site of action for the genes in the da-abo cluster.

mfs48 was initially characterized as a male and female sterile with thin bristles; hup as a maternal-effect mutant which had held-up wings. hup was mapped to the right of da and tightly linked to abo; mfs48 was deficiency mapped to the left of abo and near da. We placed mfs48 to the right of da in the following way. Recombinant $J^{+}mfs48^{+}$ chromosomes from J da / mfs48 females were progeny tested for the da allele carried. Of 23 $J^{+}mfs48^{+}$ recombinants, 19 carried da and 3 da^{+}. Thus, the gene order is J-da-mfs48, with the da-mfs48 distance probably shorter than the J-da distance. This means that mfs48 is between da and abo and thus clearly maps within the cluster.

Both hup and mfs48 are lethal over a deficiency; we have found that in addition both are recessive semi-lethals. Thus, only 25% of mfs48 and 20% of hup homozygotes survive at 25°. We have also found that the fertility of mfs48 and hup homozygous males and females is temperature sensitive. At 23° both mutants are fertile (to some degree) in both sexes but at 28.5°, males and females are sterile (mfs48 females were only tested at 25° where they were found to be sterile). We have examined spermatogenesis in mfs48 and hup males raised at 23° and 28.5°. Males raised at 23° rarely show visible abnormalities in spermatogenesis. The testes are full of cells and motile sperm are observed in large numbers in the seminal vesicle. Males raised at 28.5° have no motile sperm and show a variety of defects during the later stages of spermiogenesis. The earliest defect which we have found is at the stage just after meiosis, the clew stage (Tates 1971). Cells at this stage and later contain micronuclei as well as macronuclei (see Fig. 1). In addition we find occasional spermatid nuclei with two basal bodies