Poulson, D. F. Yale University, New Haven, Connecticut. Further cases of maternal SR in Drosophila species.

When examined in this laboratory two cases of SR found in Brazil by C. Malogolowkin proved to be spirochete associated. One was in a strain of D. willistoni from Recife referred to as Recife DI, SR 32. The other was in a strain of D. paulistorum from Belem referred to as Belem SR. Both strains were maintained at Yale for several years, but have recently proved difficult to keep. These bring to six the number of known spirochete associated cases of SR in the willistoni species group. As reported earlier there is no evidence of spirochete involvement in the SR lines of bifasciata from Italy and Japan. The same appears to be true of a new case of SR in D. robusta found in a line from Florida by H. L. Carson who has kindly provided materials for study. To date all examined cases of SR in the willistoni species group involve the presence of spirochetes while those in other species groups have given no evidence of such involvement. However, artificially transferred SR from members of the willistoni group can be maintained in very different species such as melanogaster, pseudoobscura, bifasciata, virilis, hydei, and robusta with varying levels of success depending on strain of spirochete and strain of host.

Ursprung, H. The Johns Hopkins University, Baltimore, Maryland. In Vitro hybridization of Drosophila alcohol dehydrogenase.

Isozymes of alcohol dehydrogenase (ADH) in Drosophila have recently been found independently in three laboratories (Nature 204:906, 1964; Science 149:80, 1965; J. Exp. Zool., In press). Two types of homozygous strains were found, I and II, each containing three ADH isozymes. The two strains differ in the electrophoretic mobility of at least one isozyme. A hybrid fly, III, contains seven ADH isozymes: the four parental forms and three hybrid molecules. These results are consistent with the assumption that Drosophila ADH is a dimer.

We have now succeeded in producing the same hybrid molecules in vitro. Flies of types I and II were extracted in 6M guanidine hydrochloride and the extracts combined. No ADH activity was detected in these extracts after agar gel electrophoresis and staining in a mixture routinely used for the demonstration of ADH. This inactivation is reversible however. When the combined extracts are dialized against dilute buffer, electrophoresed, and stained, seven bands are seen, corresponding in electrophoretic mobility to the seven bands of a hybrid fly.

An investigation of the mechanism(s) involved in this in vitro hybridization is in progress. Recovery of bands in the hybridization experiment is favored by β-mercaptoethanol. Guanidinium hydrochloride treatment is not the only condition following which hybridization will occur. Prolonged dialysis of a homozygous fly extract against buffer can result in the formation of two hybrid bands, each intermediate between two parental forms. This finding suggests that ADH isozymes do not necessarily reflect the presence of two polypeptide subunits. Rather, it appears possible that the multiple forms of ADH in homozygous flies are brought about by dimerization of two physical chemical variants of one only polypeptide subunit. This assumption is in agreement with the genetic evidence that the isozyme pattern difference of the two homozygous strains is inherited in a monofactorial fashion.

Courtright, J. B. The Johns Hopkins University, Baltimore, Maryland. Electrophoretic analysis of xanthine dehydrogenase mutants.

The observation that xanthine dehydrogenase (XDH) reacts with a number of different substrates (Genetics 46:1455, 1963) has been interpreted to mean that the enzyme is multivalent, has a broad substrate specificity, or represents a cluster of isozymes which may or may not share common subunits or co-factors. Specifically, the reactivity of ry^2 mutant extracts with pyridoxal suggests enzymatic activity in the absence of a ry^+ factor.

We have combined agar gel electrophoresis (J. Expt. Zool., in press) and dehydrogenase

staining using various substrates in order to assign catalytic reactions to various proteins present in the gels more precisely. The results are listed in the Table.

Stock	Substrate in staining mixture	Number of bands in electropherograms
OreR*)	xanthine, hypoxanthine, 2-amino, 4-hydroxpteridine	2 (one migrating to the anode (A) (one migrating to the cathode (C)
ry^2	"	1 (C)
ma-1	"	0
ma-1;ry	"	0
OreR*)	benzaldehyde, pyridoxal, benzyl alcohol	1 (C, identical in migration to C above)
ry^2	"	1 (C) "
ma-1	"	0
ma-1;ry	"	0

*) A different wild type stock, Bethylie, is an electrophoretic variant in the sense that the mobilities of both bands are altered.

Clearly, both ry^2 and + contain a cathodally migrating "pyridoxal" band which is absent in ma-1. Its absence in ma-1 is probably due to ma-1 itself, since sc cv dx v ma-1 male progeny resulting from crossing over in sc cv dx v f/ma-1 females lack the band also, whereas ++++f males do contain the band. Extracts of ry^2 show the band also if XDH substrates are used, at least at pH 9. At pH 8, the pyridoxal band stains very poorly. This may account for the fact that fluorometric assays for XDH, which routinely are carried out at pH 8, fail to detect more than trace amounts of activity in ry^2 extracts.

The pyridoxal reaction present in + and ry^2 is not associated with the XDH molecule, which migrates to the anode, but with a molecule of quite different charges. Both bands stain poorly in an lxd stock (kindly supplied by Dr. E. C. Keller). The results are consistent with the assumption that both ry^2 and ma-1 are structural genes, and that lxd has a regulatory function.

Schwinck, Ilse. University of Connecticut. Storrs, Connecticut. Experimental induction of additional drosopterin formation in the eyes of various Drosophila mutants.

The amount of drosopterins in the eyes of rosy (ry) and maroon-like (ma-1) mutant flies can be increased (Z. f. Naturforschung 20b:322, 1965) up to levels found in non-autonomous transplantations by incubation of "free pupae" on paper moistened with saline-phenylalanine solutions (pupae dissected from puparium 10-20 hours before eclosion). Tyrosine, dopa and equimolar concentrations of leucine, iso-leucine and alanine were not effective in the induction of this wild type phenocopy eye color. Also, a number of cofactors of phenylalanine oxidation (NAD, NADP, $NADPH_2$, folic acid, tetrahydrofolic acid, 6,7-dimethyl-5,6,7,8,tetrahydropterin) did not enhance drosopterin systhesis in the "free pupae" incubation assay. Implantation of crystals or powder of the amino acids and cofactors cited into the thorax of 3-4 day old pupae without removal of the puparium confirmed the results of the "free pupae" incubation. In contrast to the organ transplantation studies, xanthine dehydrogenase activity is not enhanced in the phenylalanine-induced drosopterin phenocopy with rosy and maroon-like pupae. Furthermore, a similar phenylalanine dependent enhancement of drosopterin synthesis was observed for mutants with normally reduced drosopterin quantities and normal isoxanthopterin accumulation (claret, ca and pink, p), again indicating a non-involvement of xanthine dehydrogenase in the phenylalanine-dependent drosopterin phenocopy mechanism.