Gelti-Douka, H., T.R. Gingeras and M.P. Kambysellis. Athens University, Greece; New York University, New York. Site of yolk protein synthesis in D. silvestris.

The observation of yolk proteins in the hemolymph of mature Drosophila species suggested that these proteins are synthesized outside the follicle as in the case of other insects (Telfer and Anderson, 1968). We attempted to localize the site of synthesis of the yolk proteins in the large Hawaiian species, D. silvestris. Tissues from single flies were dissected and washed thoroughly, initially in Waddington's Ringers and then in Grace's tissue culture medium; homogenized in borate-saline and either applied on SDS polyacrylamide gels for electrophoresis, or tested against antibodies prepared with D. silvestris crude yolk extracts. Of all the tissues used (muscles, epidermis, middle gut, malpighian tubes, fat body, oocytes and hemolymph with blood cells), only mature oocytes and hemolymph had the characteristic electrophoretic bands of yolk protein and gave immunoprecipitin lines on Ouchterlony double diffusion immuno-plates. Identical results were obtained with D. virilis. In view of these results we cultured muscles, epidermis or fat body of D. silvestris in Grace's medium containing H^3-leucine. After five hours of incubation, the culture medium was analyzed for iso-

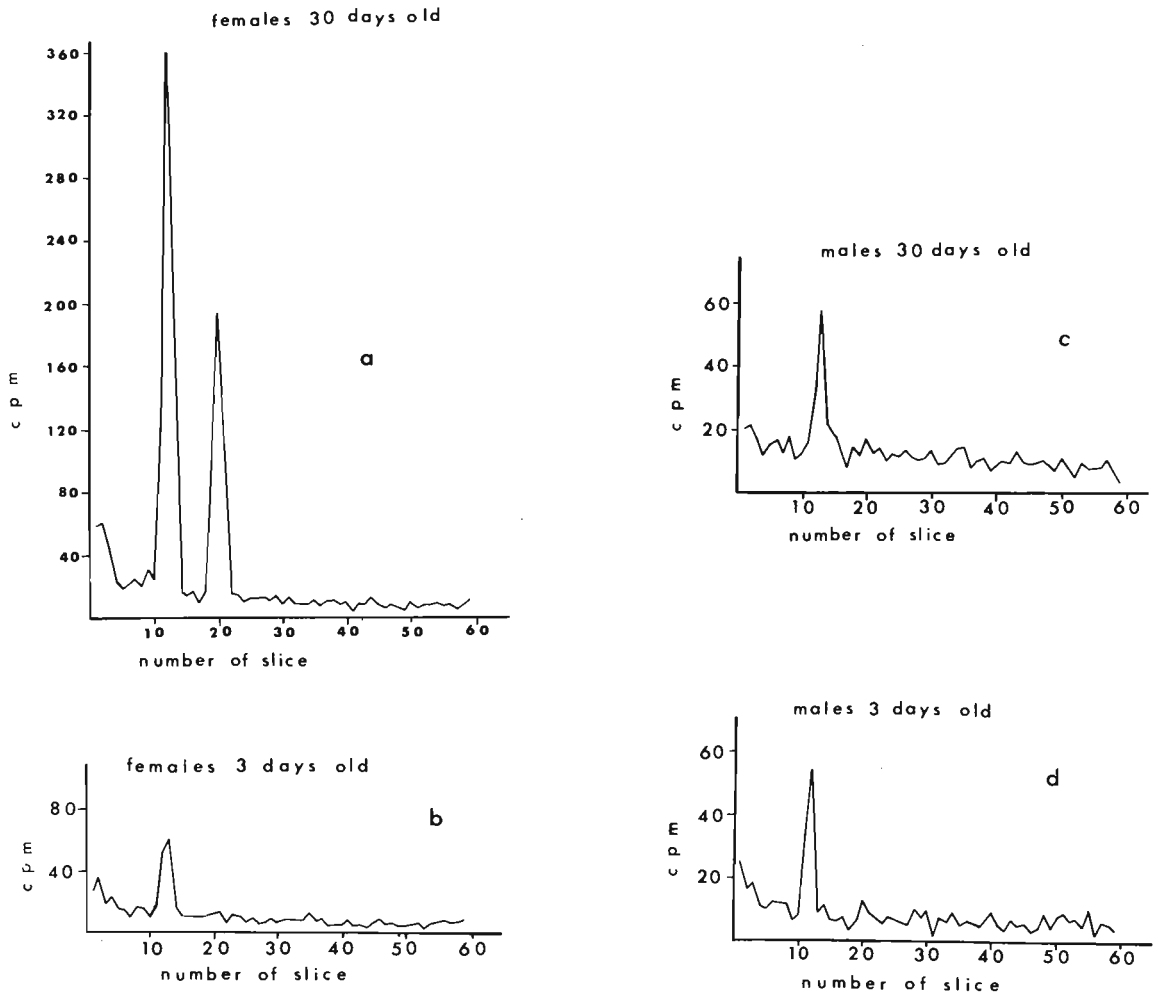

Figure 1. Fat body of D. silvestris cultured in 100 µl of Grace's culture medium for five hours in the presence of H^3-leucine (25 µC/ml). A sample of the culture medium (25 µl) was incubated for one hour with antibody prepared with D. silvestris crude yolk extract. The precipitated material was analyzed by SDS polyacrylamide gel electrophoresis. The gel was sliced into 1 mm slices and the isotope incorporation in each slice was determined by liquid scintillation counting.

tope incorporation into total protein and into antibody-precipitable yolk protein by means of liquid scintillation counting, both directly and after gel electrophoresis. The results demonstrated that the fat body from females was able to synthesize and release into the culture medium several proteins, two of which (one major and one minor) were yolk proteins (Table 1, Figure 1a). Only fat body showed detectable synthesis of yolk proteins. Using fat body from newly emerged female flies (3 day old) we found that only one yolk protein was produced. The other yolk protein appeared during or near adult maturation (Figure 1b). The exact time of its synthesis has not been determined as yet. Fat body from males produces only small quantities of the major yolk protein (Figure 1c, d). From these results it is clear that the yolk proteins in Drosophila species are synthesized in the fat body, released in the hemolymph and taken up by the oocytes. This last step appears to be mediated by juvenile hormone (Kambysellis and Heed, 1974). (Supported by NSF Research Grants GB-29288 and GB-34168.)

Table 1. Fat body of D. silvestris cultured in 100 µl of tissue culture medium for six hours in the presence of H^3-leucine and H^3-phenylalanine.

Fat body	TCA precipitated proteins (cpm)	Antibody precipitated proteins (cpm)
females	7,586	2,026
males	931	878

References: Telfer, W.H. and L.M. Anderson 1968, Devel. Biol. 17:512; Kambysellis, M. P. and W.B. Heed 1974, J. Insect Physiol. (in prep.).

Bortolozzi, J., R.C. Woodruff and T.K. Johnson. University of Texas, Austin.

Frequency of spontaneous lethal mutations in darkness in Drosophila melanogaster.

A preliminary experiment (Woodruff and Bortolozzi, 1973) suggested that the absence of light may increase the frequency of spontaneous sex-linked lethal mutations in D. melanogaster. To test this hypothesis, the following experiment was performed.

Untreated Oregon-RC males 3 days old were pair mated with FM7, y^{31d} w^a 1z v B/sc10-1 virgin females on standard corn meal medium. These matings were divided in two groups. One group (light) was kept under standard laboratory conditions in the light for about nine hours in 24 hours and the other group (dark) was kept in the dark for the entire experiment.

The F_1 FM7/Oregon-RC virgin females were backcrossed with FM7 males and F_2 offspring were scored for the absence of Oregon-RC males. The results of this experiment are summarized in Table 1.

The analysis of Table 1 shows that the frequency of lethal mutations in the dark is significantly higher at the 1% level (Stevens, 1942) than the frequency in the light. The reasons for this increase in spontaneous mutations are unknown. We are currently performing complementation tests with all of the lethal mutations recovered in this experiment. This will enable us to determine if any lethals belong in clusters and to determine if nondisjunction is involved.

Dark	Light
11/1,741 = 0.63%	4/1,862 = 0.22%

Table 1. Frequency of spontaneous recessive sex-linked lethal mutations in the dark and in the light.

It is of interest to speculate that D. melanogaster may have a light dependent repair system. In the dark, this system would be inoperative and would lead to an increase in the frequency of unrepaired mutations. This possibility is being currently investigated.

References: Stevens, W.L. 1942, J. Genetics 43:301; Woodruff, R.C. and J. Bortolozzi 1973, DIS 50.

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