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A replica technique for the electron microscopic analysis of the wing surface of Drosophila.

A two stage replica procedure has been devised to allow fine structure observation of the wing surface. The technique would be valuable for studies on speciation, phenogenetics, and cuticle development. The method is as follows: 1. Rinse entire wings in 70% ethanol and dry. 2. Place wings on a methyl methacrylate plate* (methyl methacrylate, 98%; benzyl peroxide, 2%; heat at 80°C. until polymerized), sandwich between two clean glass slides, and fix in place with an even, firm pressure (two ordinary paper clamps will do). 3. Heat to 100°C. for 30 to 40 mins. and cool to room temperature. 4. Remove the glass slides and cover the methyl methacrylate with 10% polyvinyl alcohol (dissolved in double distilled water at 80°C). Allow the alcohol to dry and strip off the residue with forceps. All wing remains should be removed by 4 to 5 repetitions of this procedure. 5. Shadow the methyl methacrylate cast with carbon (200Å) and chromium (50Å). 6. Using a sharp razor, cut the metal film into squares small enough to fit on a grid (3mm. x 3mm.). 7. Place the shadowed methyl methacrylate into a bath of 1:1 chloroform-benzene which slowly dissolves the plastic and releases the metal film. Pick up the film on a grid and wash 3 to 4 times in a fresh preparation of the same solution. 8. Observe in the electron microscope.

Fig.1. Upper surface of the wing of a Canton S female. Magnification 10,000X black line = 1 micron. Note the regular granular structures on the wing surface. Each granule is marked by a center pore. The base and shaft of wing hairs are evident.

The photograph illustrates some of the regular detail visible in such a preparation.

*Commercially available from Oken-Shoji Co.,Ltd., Katagiri Bldg., Ginza Higashi, Chuo-ku, Tokyo, Japan.

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Alleaume, Nadine. Division of Biology, California Institute of Technology, Pasadena, California. Vital staining of Drosophila eggs.

Flies injected with trypan blue in 0.4% Drosophila ringers solution lay eggs with blue yolk (K. Sander and H. Vollmar). We found feeding to be more convenient than injection for producing stained eggs. Adult wild-type flies were fed for three days on medium containing either Nile blue (0.01%), Toluidine blue (0.02%), Bismark brown (0.5%) or N Phenyl Nile blue chloride (0.05%). The flies were transferred to dye-free medium and about half of the eggs they laid in the following two days were found to contain dye. The first three dyes colored only the yolk. N Phenyl Nile blue chloride led to light pink eggs with pink fat bodies in the developing embryos. With these four dyes the embryos in stained eggs develop normally.