A simple nail polish imprint technique for examination of external morphology of *Drosophila* eyes

In view of the ever-increasing genetic information and other experimental strategies, *Drosophila* continues to be a useful model for studying the various developmental pathways and genetic networks that regulate these events[1,2]. In recent years, fly models for different human neurological and other genetic disorders have also been developed and these have been widely used for identifying interacting genes and for developing therapeutic strategies[3]. The compound eyes of flies have been particularly useful in this context because (i) the genetic pathways that determine eye development are better understood, (ii) the adult eyes contain both neuronal and non-neuronal cell types, (iii) they are dispensable for survival of the flies, and (iv) the phenotypic consequences of any perturbations in eye development are distinct and quantifiable[4]. The adult *Drosophila* eye contains about 800 ommatidia arranged in a highly ordered matrix with arrays of sensory bristles projecting out from the surface of each ommatidial unit[5].

Most studies exploiting these features of the *Drosophila* eyes use scanning electron microscopy (SEM) to examine any disruption in the ordered arrays of the ommatidia in adult eyes. We describe here a novel, simple and inexpensive method that provides high quality images, comparable to those obtained by SEM, of the external surface of eyes of adult flies, but which does not require expensive facility and can be used in any laboratory with a good light microscope.

In this method, we use a transparent nail polish to create an exact replica of the external surface of the eye, which is subsequently examined using a light microscope. To obtain an imprint of adult eye, a small drop of transparent nail polish is placed on the surface of a clean glass slide. The fly to be examined for eye morphology is anaesthetized, placed on a dry area of the slide and decapitated with a sharp blade or needle. The decapitated head is held with forceps or needles and is briefly dipped in the still fluid drop of nail polish. The head is then placed in a clean and dry area of the same slide and the nail polish layer on the eyes is allowed to dry at room temperature (24°C) for 5–10 min. The dried layer of nail polish can be easily peeled-off from the eye with the help of fine dissecting needles. The separated peel, being an exact replica of the eye surface, assumes a goblet-shaped appearance. This peel is carefully placed on another clean glass slide with the imprint side facing upright. This nail polish imprint can be directly examined and photographed under a stereobinocular microscope to provide a low magnification image of the eye surface (Figure 1 *a*). For higher magnification and better-resolved image of the eye surface, the peel is carefully flattened by gently placing a cover slip over it and carefully applying a slight pressure. The eye imprint is then examined under a microscope using 10X (Figure 1 *b, g, j*), 20X (Figure 1 *d, h, k*) or 40X (Figure 1 *e, i, l*) differential interference contrast (DIC) objective. These can be seen with bright field optics as well, but the DIC optics provide a better image. As may be noted from Figure 1, details of eye surface are generally comparable with those obtained by SEM (Figure 1 *c, f*). As shown in Figure 1 *g–l*, the nail polish imprints also easily reveal the characteristic defects due to different mutants affecting eye development. This method can be applied to study surface details of compound eyes of any insect and possibly other cuticular structures from which the peel of nail polish can be easily removed.

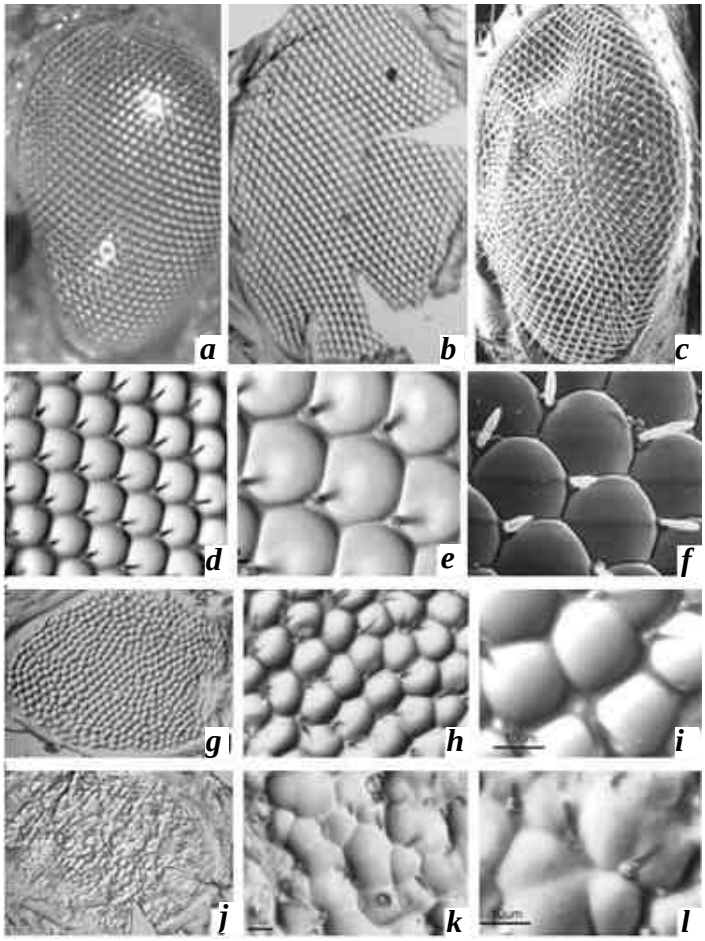

Figure 1. Nail polish imprints (***a***, ***b***, ***d***, ***e***, ***g–l***) and SEM images (***c***, ***f***) of eyes of adult *Drosophila*. Images in (***a–f***) are from wild type, in (***g–i***) from *Elp-B1/CyO* and in (***j–l***) from *GMR-argos* mutant flies. The image in (***a***) was taken with a stereobinocular microscope (Zeiss Stemi SV6) using a 5X objective; in (***b***), (***g***) and (***j***) with a 10X objective; in (***d***), (***h***) and (***k***) with a 20X, and in (***e***), (***i***) and (***l***) with a 40X objective using a Nikon Ellipse 800 DIC microscope.

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RICHA ARYA
S. C. LAKHOTIA*

Cytogenetics Laboratory,
Department of Zoology,
Banaras Hindu University,
Varanasi 221 005, India
**For correspondence.*
e-mail: lakhotia@bhu.ac.in