Lin, J. Department of Biological Sciences, Northwestern State University, Natchitoches, Louisiana, U.S.A. A polytene chromosome arms spreader.

To get a good spread of the larval salivary gland polytene chromosome arms by squashing method (Ashburner, 1989) is often beyond our reasonable control. There are many factors influencing the outcome of a spread which include the age of the larvae, strain of the insect, amount of fat bodies associated with the glands, the composition and temperature of the isolation solution, and most important of all, the strength and direction of tapping onto the coverslip of the preparation.

This paper describes an alternative method. The distinctive feature of this method is to offer a way to hold the coverslip and tossing the chromosomes up and down for the arms to be spread by capillary force. Again, the success of getting good spreads depends on the strength and frequency of tossing. I obtained good spreads more frequently with this device than with the conventional method.

The spreader I designed is made of small pieces of 1/4" and 1/16" thick plexiglass. The thickness is not critical. It can also be constructed of small pieces of wood. A 10 cc or 12 cc plastic syringe, a rubber cap of a Vacutainer blood collecting tube, a large paper clip, a used 2" × 2 1/2" photographic film or cut out from a plastic folder cover, and a small amount of superglue are the additional materials for the construction.

The different parts of the spreader in Figure 1 are prepared as follows:

a. Syringe plunger: A hole is drilled at the upper inner corner of one of the four fins of the syringe plunger with a needle flamed on a burner. The size of hole should only be slightly larger than the diameter of the wire of a large paper clip.

b. Plunger holder: A large paper clip is bent straight with fingers. A 1 1/2" section is cut out with a wire cutter, and one end is slipped in with a 3/8" length of insulation sleeve cut out from an electric wire. After threading the paper clip wire through the hole in the syringe plunger, another piece of insulation sleeve is slipped into the other end of the paper clip wire. The insulation sleeves serve the function of limiting the side travel of the plunger holder. The ends of the plunger holder are bent up as shown.

c. Body of the syringe: If the syringe you use has a colar outside the needle connecting end, you should cut it off with a sharp single side razor blade.

d. Syringe guide. A clear plastic film of 2" in length and 2 1/2" in width is wrapped around the syringe body. The overlapping ends are glued with small amount of superglue. After the syringe stand is constructed, the syringe guide will be glued to parts e and f at the edges of the large central holes.

e. Upper end of the syringe stand: A 1 1/2" × 3" × 1/4" plexiglass piece is marked in the center with a ring with a diamond pencil. The diameter of the ring should be about the same as the diameter of the syringe guide. Use flamed hot needle drill holes along the ring and remove the central piece. Smooth the central hole with a round file. Be sure the syringe guide can snuggly fit through the hole.

f. Cental board of the syringe stand: A 1 1/2" × 3" × 1/8" plexiglass is drilled with a central hole same as "e" above. The four corners of the board are notched (1/4" × 1/2" pieces) to fit to the four pillars of the stand.

g. Pillars of the stand: four 1/4" × 1/2" × 2" plexiglass pieces are cut and all sides smoothed with sand papers.

h. Bottom board of the stand: A 1 1/2" × 3" × 1/4" plexiglass without central hole or corners removed.

i. Vacutainer cap: The rubber cap of a Vacutainer blood collecting tube with a diameter of the large end slightly smaller than the diameter of the syringe body is bored with a small hole in the center with a cork borer. The syringe tip is poked through the hole in the rubber cap. The top of the rubber cap is glued to the end of the syringe body with superglue.

j. Coverslip: It is good to use siliconized round coverslip of 8 mm in diameter, which is close to the diameter of the small end of the rubber cap. The coverslip of 20 mm in diameter as shown in the photo is just for showing how the coverslip is sucked up at the end of the rubber cap, it can not be visible in the photo if a 8 mm diameter coverslip is used.

After all parts are prepared, the plexiglass parts and the syringe guide are glued together with superglue.

In practice, a microscope slide loaded with two to three pairs of salivary glands dissected from the third instar larvae is added with two drops of polytene chromosome pre-treatment solution (Kalish and Whitmore, 1986). The rubber cap of the syringe, which has been cleaned with 70% ethanol and dried, is pressed against the coverslip on a flat surface. With the plunger holder moved to the side and the plunger inserted all the way into the syringe, the plunger is pulled up quickly. Dropping of the plunger holder to the top of the syringe body to hold the plunger is automatic. The coverslip is held snuggly by vacuum with the center of the slip slightly bent in. The glands on the slide are then placed on the base of the syringe stand under the syringe guide from the side of the syringe stand (shown by arrow in Figure 1). The syringe assembly is inserted into the syringe guide down to the glands. Move the syringe assembly up and down gently for five to six times and exam the specimen under a phase microscope. Repeat the process if not enough spreading of the arms of

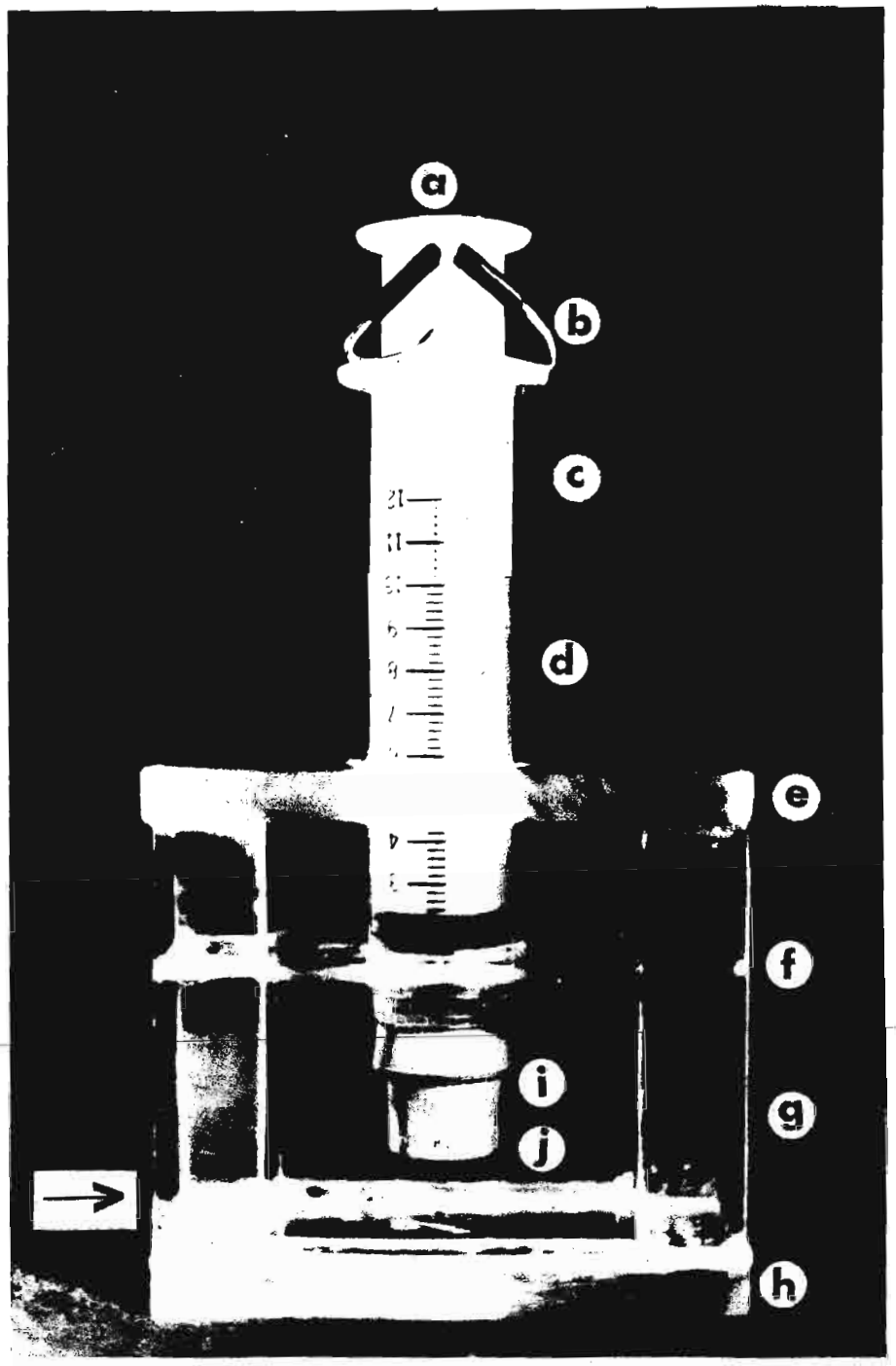

Figure 1. A polytene chromosome arms spreader.

chromosomes is obtained. If the spreading is satisfactory, drop the coverslip onto the microscope slide by moving the plunger holder to the side to break the vaccum. Further processing of the glands, such as removal of coverslip by freezing with liquid nitrogen and staining with special stains, will depend on the purpose of the investigation.

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References:

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