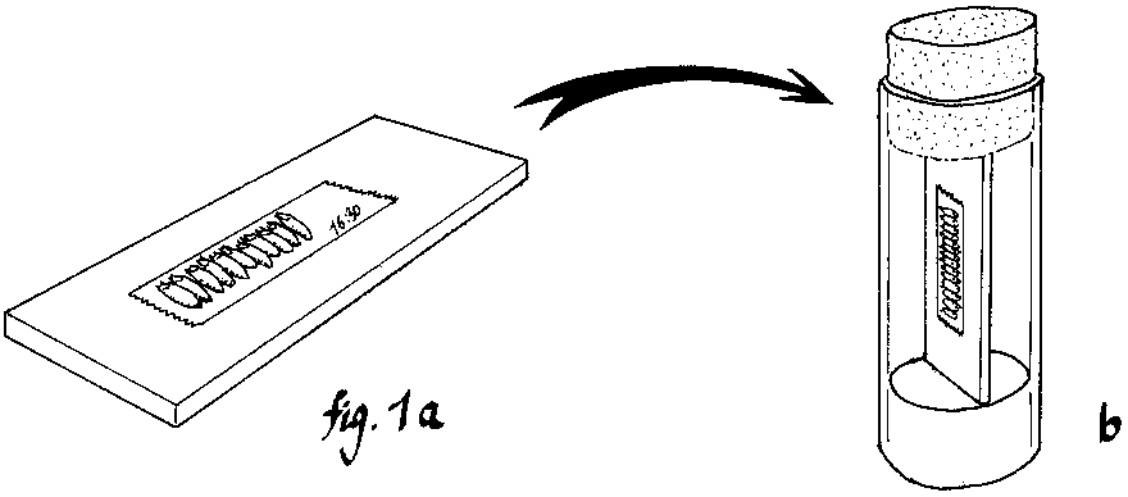
Labelling *Drosophila* pupae with BrdU

ITO Kei, 1992 October

COLLECTING STAGED PUPAE

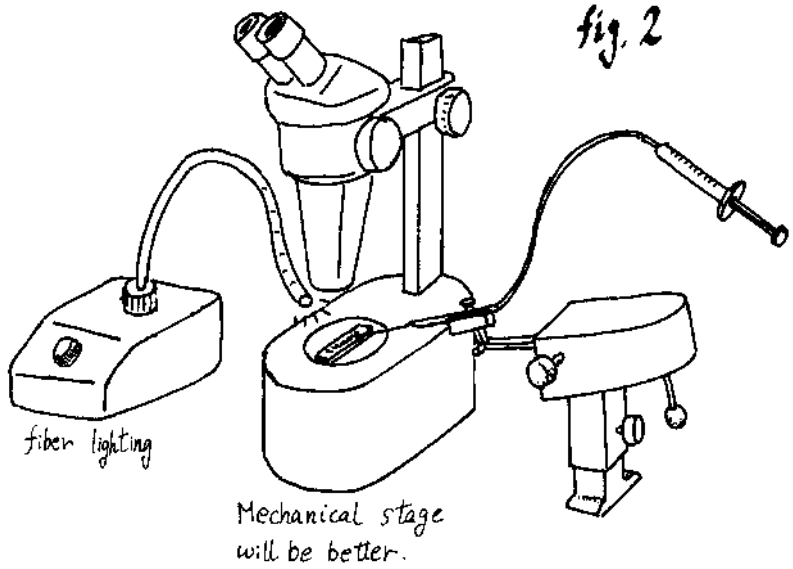
1. Prepare a couple of bottles containing late 3rd instar larvae, which wander on the botlle wall (110-120 hours after egg laying at 25°C), and remove all the pupae already attached to the wall.
   To remove pupae easily, dip a small brush (the same one people use for separating flies under dissecting microscope) into water, and touch the pupae with it; thus putting a small amount of water around the pupal case. The glue of pupae will dissolve within a minute. Then take the pupae with the same brush. Do not try to take pupae with forceps; the pupal case is very fragile.
2. Cut about 3 cm of dubble-sided tape (such as Scotch Tape) and put it onto a slide glass (Fig. 1a).
3. Check the culture bottles every one hour. Take white prepupae, which no longer moves, with the wet brush, and put them onto the slide glass, dorsal on top (Fig. 1a).
4. After collecting 10-20 prepupae, write the time of collection onto the tape.
5. Put the slide glass into a new bottle containing normal fly medium (Fig. 1b). The medium will provide enough humidity for the pupae. A humid chamber with wet paper is sometimes too "wet" so the Scotch Tape becomes non-sticky.
6. Repeat steps 3 to 5 until you collect all the prepupae in the bottles. Keep the bottles at certain temperature (typically 25°C) and let the pupae continue developing.
7. Drink some coffee or tee and do the job again after one hour.

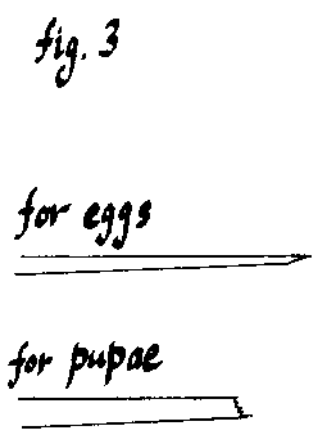


INJECTING BrdU INTO PUPAE

1. Prepare a micro manipulator and a high magnification dissecting microscope as in the Fig. 2. As pupae are quite big and not transparent, normal light microscope is not good for this purpose.

2. Make an injection needle by pulling a capillary with a needle-puller for electrophysiology or P-element injection (See *"Drosophila, a practical approach"*, IRL Press, pp. 165 and 193). You can use the same capillary as the one for the P-element injection.
   Set the needle just after pulling to the micro manipulator and connect it to a 1-ml syringe with air-filled plastic tubing (Fig. 2); any other kind of injection apparatus (oil-filled tubing, Pico-Injecter, etc.) will also be OK.



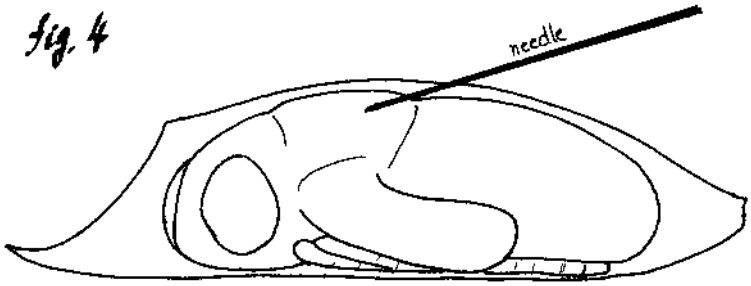


3. Break the tip of the needle with fine forceps under the microscope. Since the needle must penetrate thick pupal cases, the tip should be rather thick and robust (Fig. 3). Grinding the tip is not needed.

4. Put a few microlitter of BrdU solution onto Parafilm (or equivalent plastic film) under the microscope. Dip the tip of the needle into the drop and gently pull the syringe so that the solution goes into the needle.
   The concentration of BrdU is about 3 mg/ml in PBS or ringer. The animal will continue development apparently normally after the injection, but they would not hatch because of certain side effect of the high concentration of BrdU.
   If you want to see the labelling in the adult flies, or if you want to be sure about the normal development, you can dilute the BrdU up to 0.1 mg/ml. It will then be more difficult to get a good antibody staining, however.

5. Take the slide glass with pupae of appropriate stage. Clean the surface of pupae by pouring 70% Ethanol onto them, and wait for a while until the surface becomes dry (just like doctors do before injecting their patients).

6. Put the needle into the dorsal thorax region (Fig. 4).
   Push the syringe and inject a small amount of BrdU solution. In order not to severely destroy the tissue, inject as small amount as possible.



The dorsal half of the thorax is occupied with the flight muscle; destroying the structure of this region to some extent is not harmful for the survival of the animal (Injected solution will make a small "bubble" in the muscle structure). On the other hand, the survival rate is much lower when injected into abdomen or head.
I am unfortunately not completely sure about whether the injection into thorax has no effect on the wing development.

*** You can check the stage of pupal development by comparing your animals with the descriptions by Bainbridge and Bownes (1981, *J. Embryol. Exp. Morphol.* **66**: 57-80) and by Bodenstein (1950, *"Biology of Drosophila"*, pp. 420-534).

7. Keep the slide glass of the injected animals in a bottle with normal medium for more than one hour until cells incorporate enough amount of BrdU.

8. Transfer the pupae into a shallow dish filled with PBS.

9. Remove the pupal case with fine forceps.

10. For the whole-mount preparation, dissect the body and isolate the tissue of your interest.
For the paraffin sections, cut out parts of the body which are not needed for the study, such as abdomen, legs, mouth parts of the head, and so on. This is in order to make holes through which the fixative goes into the body. It is convenient to use small scissors (Fig. 5).

11. Transfer the tissue (or body) into fixative.

fig. 5