

After running "EXPFONT" with the printer on, seven keys will be redefined from the standard font:

STANDARD:	!	@	#	\$	%	&	~	;	\
EXPFONT:	♀	β	σ	\$	%	α	°	σ	μ

DATA statements can be chosen so that any nine of the 14 non-standard characters defined here are available simultaneously. Key redefinition does not interfere with other signals from BASIC that control print pitch or quality.

Duttagupta, A.K., M. Das (Mutsuddi) and D. Mutsuddi. University of Calcutta, India. The maintenance of the sensitive *Drosophila* stocks in laboratory culture.

Perhaps all the *Drosophila* workers in the tropical countries, like India would agree with us that the transshipment of different *Drosophila* species/strains to such places is a quite difficult job. In India, the mild winter in our place, which stays from middle of November to early February, is only suitable for

receiving stocks in healthy condition. Even within this period, the stocks very often arrive either dead or with a few larvae and pupae on decomposed culture medium, often infected with fungi. Furthermore, due to mite infection and elevated temperature during the transshipment, the newly arrived flies do not give enough progeny. For these reasons, we had to find out some means to overcome the problems.

Sensitive stocks like *D.miranda* are difficult to maintain. Due to its high sensitivity to temperature (it prefers temperature below 18°C), the maintenance of the stock was initially almost a failure. The flies, at that time, used to lay very few eggs on the standard *Drosophila* culture medium which invariably developed scums; with poor hatching of the larvae, most of the eggs were destroyed before hatching. We overcame this by adopting the following method:

1. The flies were first allowed to breed on a vial containing pasted banana. A few pieces of filter papers (with nepazine) were inserted leaving some portion outside the food. The flies were observed to lay eggs more in number. The eggs hatched successfully and the larvae were rather healthy.
2. At pupation (meanwhile the banana gradually became decomposed) the pupae were brought outside the vial and washed carefully in *Drosophila* Ringer (pH 7.2) to remove the decomposed food.
3. The pupae were dried on filter paper.
4. The empty vials were taken and their inner walls were smeared (with brush) with *Drosophila* culture medium to make the wall sticky. The pupae were applied to stick there by placing their ventral sides facing to the wall of the vials.
5. The newly emerged flies were transferred to the fresh culture medium.

By following the same method, we were also successful in removing the mites from *Drosophila* stocks. The pupae were brought outside the mite-infected culture medium and were washed in Ringer carefully and the above described process was followed to make mite-free stocks.

Hey, J. and D. Houle. University of New York, Stony Brook, USNA. Rearing *Drosophila athabasca*.

Drosophila athabasca is a complex of three semispecies (Miller & Westphal 1967), all of which are difficult to rear in the laboratory. We have developed a media and rearing protocol that works well for

these flies and allows their use as a convenient experimental organism. The basic food recipe is a simple modification of the standard *Drosophila* media and consists of the following: 2900 ml of water; 116 gr of corn meal; 116 gr of dead brewer's yeast; 80 ml of molasses; 80 ml of light corn syrup; 24 gr of agar; 15 gr of peptone (SIGMA catalog no. P-7750); 15 gr of casein (SIGMA catalog no. C-0376); and 35 ml of 10% Tegosept in 95% ethanol. Whenever wild caught flies are used, we also add 0.6 gr of streptomycin sulphate and 0.2 gr of penicillin.

The ingredients could probably be combined in a variety of ways, though we prefer the following procedure: bring 2200 ml of water to a boil; mix the agar with 200 ml of water and quickly add it to the boiling water; bring the mixture to a boil while stirring; remove from heat and add the corn syrup and molasses; combine the remaining water with all of the remaining solid ingredients by stirring in a blender at high speed for several minutes; add this slurry to the water/agar/sugar mixture and stir to homogeneity;

the tegosept is added last. The food is pumped while hot in 7-8 ml aliquots into 8 dram vials, and is sufficient for nearly 500 vials. Great care must be taken to avoid the introduction of any molds or yeasts to the food, so we generally cover the vials with sterile cheese cloth while they cool before plugging with sterile cotton plugs. The critical way that this food differs from others appears to be the addition of the peptone and casein. It is quite possible that different sugars or different proportions of other ingredients may also be satisfactory.

For rearing, all flies are kept in the light at 19° and 80% humidity. A long photoperiod or constant light is important because these flies seem to go into diapause with a short photoperiod (pers. obs.) and will not mate in the dark (Curtright & Miller 1979). When the larvae in a vial begin to pupate, a small square of sterile tissue is placed inside the vial and dampened with a few drops of 1% propionic acid. If this tissue is not added as a pupation site, nearly all larvae will pupate in the food and die. If flies are young when they are put in a vial of new food, they often will not produce many eggs for several days. In this time the food may dry out or develop a yeast culture. We often find it preferable in these cases to simply transfer the flies to a vial of fresh food.

With these methods we have found *D.athabasca* to be quite tractable. Wild caught females successfully produce progeny greater than 90% of the time and any particular line can be maintained indefinitely if 3 or 4 vials are maintained in rotation. We have also found these methods suitable for other related species including: *D.algonquin*, *D.affinis*, *D.narragansett*, *D.pseudoobscura*, and *D.azteca*.

References: Miller, D.D. & N.J. Westphal 1967, *Evolution* 29:531-544; Curtright, R.D. & D.D. Miller 1979, *Anim. Behav.* 27.

Marcus, C.H. Albert Einstein College of Medicine, Bronx, New York USNA.
Single fly DNA extraction procedure.

In order to obtain usable DNA from single flies, we developed the following straightforward procedure. There are two distinct features of this procedure. First, there are no precipitation steps. Second, homogenization occurs directly in the microfuge tube

in which the fly is stored (see Figure for how to make homogenizer), thereby eliminating the need to transfer sample from a standard homogenizer (DNA sticks to glass), and the time consuming process of washing standard Dounce homogenizers after each extraction.

1. Homogenization. (a) Take frozen fly in microfuge tube and add 50 microliters of ice cold homogenization buffer (-SDS) (0.15 M NaCl; 0.015 M EDTA, pH 8.0; 0.05 M Tris, pH 8.0). Note: we like to keep frozen flies on dry ice up until homogenization buffer is added. (b) Using the pasteur pipet homogenizer (see Figure) grind fly for 30-45 seconds using a twisting motion (as opposed to a piston-like motion, to prevent possible shearing). Most fly parts should be homogenized with possible exception of wing and leg parts. Eye pigment if present is a useful indicator of complete homogenization. (c) With homogenizer still in microfuge tube rinse the homogenizer with 50 microliters of room temperature buffer (+SDS) (same buffer as above with 0.04% SDS). We feel that the SDS concentration is critical. Higher concentrations of SDS have proved to inhibit restrictability of DNA.

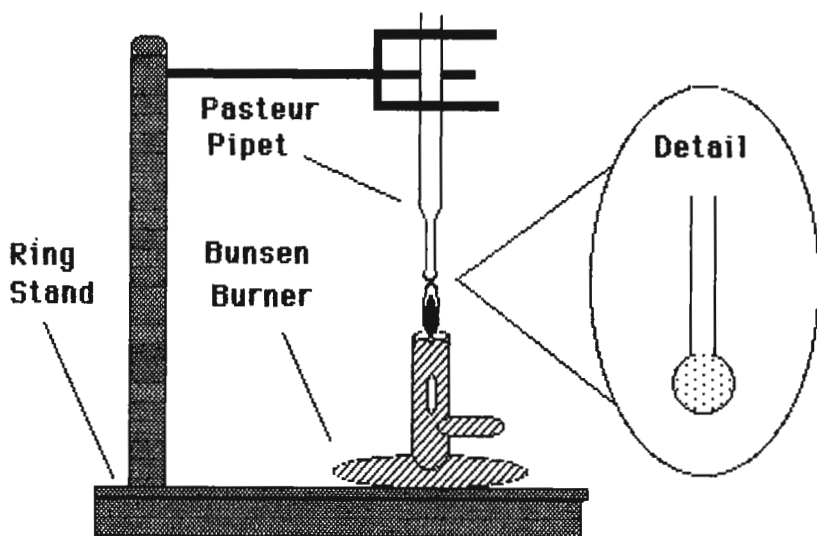


Figure. Making homogenizer: using above assembly, heat pipet until a solid ball of a diameter of approx. 3 mm is reached. Homogenizer should fit neatly into bottom of a microfuge tube.