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; Curtwright, R.D. & D.D. Miller 1979, Anim. Behav. 27.

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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.

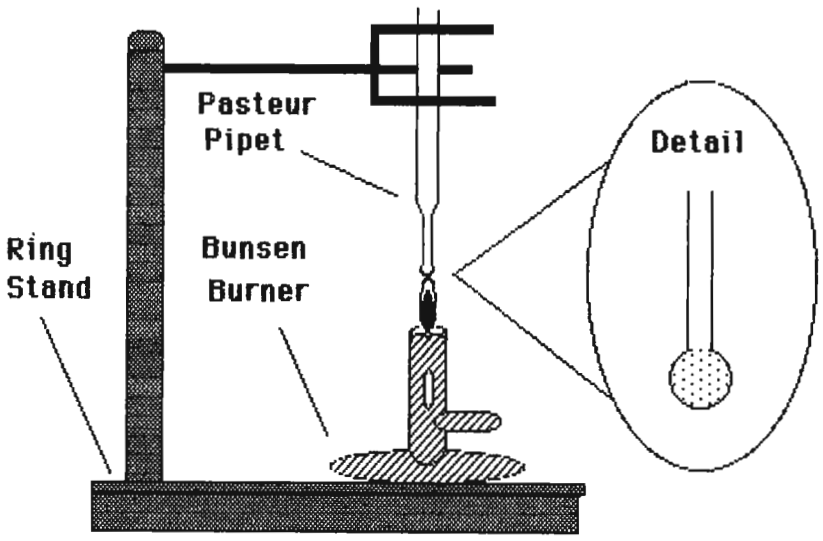

Figure. Making homogenizer: using above assembly, heat pipet until a solid ball of a diameter of approx. 3 mm is reached. Homogenizer should fit neatly into bottom of a microfuge tube.

2. Proteinase K digestion. Immediately add 5 microliters of ice cold Proteinase K (10 mg/ml) and place at 65°C for 30-60 min.

3. Phenol:chloroform extraction (2X). (a) Add 200 microliters of1:1 phenol:chloroform solution. Phenol is buffered with TE (10 mM Tris HCl, pH 8.0; 1 mM EDTA, pH 8.0). (b) Mix phases thoroughly by gently inverting tube 5 or 6 times. Microfuge for 3 min (first extraction), 2 min (second extraction). (c) Take top layer using a cut pipet tip. Use cut tips whenever handling genomic DNA to prevent shearing of DNA. (d) Repeat (a) - (c).

4. Isoamyl alcohol extraction. (a) Add 200 microliters of isoamyl alcohol. (b) Microfuge briefly (2-3 seconds). (c) Discard top layer, which is isoamyl alcohol.

5. Dialysis. (a) We use spectrapor membrane tubing 1.0 cm, molecular weight cutoff: 12,000-14,000. We boil tubing in approx. 5mM EDTA, pH 8.0 twice, and then store tubing in 50% ethanol. (b) Take sample (with cut pipet tip) and place in dialysis bag--seal bag. (c) Dialyze against TE. We use roughly 1500-2000 fold volume for 36-48 hr, changing solution four times. (d) Remove DNA samples.

Procedure can work just as well for ten flies using the same procedure, but raising volumes as follows: homogenization buffer -SDS: 200 microliters; homogenization buffer +SDS: 200 microliters; Proteinase K (10 mg/ml): 20 microliters; Phenol:chloroform: 800 microliters; Isoamyl alcohol: 800 microliters. Note: for multiple extractions a single homogenizer can be used. After each homogenization wipe homogenizer, dip homogenizer in pure ethanol, wipe, dip in distilled water, wipe.

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A method for observing the behavior of groups of flies.

We have developed a simple procedure for observing the behavior of groups of flies. This technique has been used to study courtship, although it could be used to study any behavior that flies in a group perform.

The observation chamber is a square plastic petri dish (Falcon 1012, 100 x 100 x 15 mm) into which a thin layer of cornmeal-molasses-agar medium has been poured. After hardening, a small section of medium is removed from two opposing corners of the dish so that flies can be introduced without their sticking to the food. The flies are transferred to the chamber by aspirating them through small holes in the lid, which have been made with a soldering iron, that are over the corners from which medium has been removed. In our study the flies were anesthetized with CO_2, although it would be easy to introduce un-anesthetized flies into the chamber. The entrance holes are then covered with clear tape to prevent flies from escaping. A light is positioned over the chamber and the behavior of the flies is monitored by observation through the lid.

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Milner, M.J.. University of St. Andrews, Fife, Scotland. Culture medium parameters for the eversion and differentiation of **Drosophila melanogaster** imaginal discs in vitro.

We use Shields and Sang's M3 (Shields & Sang 1977) for the culture of imaginal discs in vitro. Originally, the 1977 formulation supplemented with 10% non-heat inactivated foetal bovine serum (FBS) was used, but more recently we have reduced the amount of FBS to 2%, as this yields better differentiation. This necessitates a non-serum formulation of the medium (Table 1) to compensate for the absence of various ions previously supplied via the higher level of serum (Shields & Sang's M3(NS) - Shields & Sang, pers. comm.). It should be noted that this formulation is also bicarbonate-free. The medium is made up as before except that the pH is raised directly to 6.8 by addition of 1% NaOH. A batch of medium may be used for up to 6 weeks after preparation, and a dilution series of 20-hydroxy ecdysone is best used within 10 days of preparation. As found for embryonic cell culture, optimal medium conditions are reached between the first and second week after serum addition (Shields & Sang 1977).

It may be desirable to culture discs in the absence of serum, either because of difficulty in obtaining a suitable batch of serum, or in order to culture discs in more rigorously defined medium conditions. To this end, I have tested a number of serum substitutes used in other tissue culture systems, at a range of concentrations, adding them directly to M3(NS) and assessing their ability to support eversion, differentiation and pigmentation of wing discs on a scale of 1 to 3. One represents poor differentiation, 3 good development and 2 an intermediate level. All additives were purchased from Sigma. The results