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Cryopreservation of whole flies, a technique for the preparation of genetic voucher specimens.

Hanner, Robert, and Brian Webster[1]. Curatorial Associate, American Museum of Natural History, Central Park West @ 79th St. New York, NY 10024; Phone: (212) 769-5663; Email: hanner@amnh.org; [1]Current Address: 012 Baker North Hall, Cornell University, Ithaca, NY, 14853; Email: brw26@cornell.edu

The traditional focus of museum collections has been on preserving organisms for morphological studies. In the last several decades, it has become clear that the information content of biomolecules themselves make them especially worthy of preservation. Freezing remains the most reliable and versatile method of preserving tissue for long-term storage (Engstrom, *et al.*,1999), while ultracold storage of biological tissues preserves a broad suite of biochemical characteristics (Cook, *et al.*, 1999), although protein and lipid changes can occur during frozen storage (Florian, 1990).

To make specimen-based collections of biological relevance to current and future genetic research techniques, researchers need to consider optimizing their collection and preservation procedures. Treatment of specimens with preservatives used in anatomical studies, or with solvents such as ethanol, allows degradation of DNA and is not recommended. Storage of homogenates or extractions are likewise problematic as they are often unstable over time and because mechanical manipulation of tissues during homogenization and extraction fragments DNA.

Here we present a minimally invasive method for the cryo-fixation of whole flies. This method utilizes the best available science to establish a collection of voucher specimens that can anticipate the unforeseen needs of future researchers by preserving tissues of the highest possible quality without the use of potentially damaging preservatives. The objective is to achieve a controlled rate of freezing, as opposed to the common practice of flash freezing specimens in liquid Nitrogen. The inherent simplicity of the protocol should make it a valuable tool for both field and laboratory researchers alike. An added advantage is that one the specimens are fixed, the specimen collection apparatus can also be used to ship the specimens by air freight.

Materials Required

-Isopropyl alcohol
-1.8 ml cryo-vials (preferably with gasket lids, available from Nunc and others)
-Cryo 1ºC Freezing Container (available from Nalgene, Catalog # 5100-0001)
-dry cryoshipper large enough to accommodate the freezing container (such as the MVE model CE, which can hold up to 3 freezing containers at a time).

Procedure

Following the manufacturers instructions, charge the cryoshipper with liquid Nitrogen and charge the freezing container with ambient temperature isopropanol. Place live flies into the cryovials and tightly close the vials. Next, place the cryovials with the flies to be preserved into the Cryo 1ºC Freezing Container and close the lid. Then, simply place the Freezing container loaded with cryovials of

specimens into the cold cryoshipper, close the lid on the cryoshipper and let the specimens equilibrate for at least three hours. The cryoshipper can then be dispatched to the ultimate repository where the specimens will be accessioned.

References: Cook, J.A., G.H. Jarrell, A.M. Runck, and J.R. Demboski 1999, The Alaska Frozen Tissue Collection and associated electronic database: a resource for marine biotechnology. OCS Study MMS 99-0008. University of Alaska, Fairbanks; Engstrom, M.D., R.W. Murphy, and O. Haddrath 1999, In: *Managing the Modern Herbarium: an Interdisciplinary Approach.* (Metsger, D., and S. Byers, eds.). pp 315-330. Elton-Wolf, Vancouver; Florian, M.L., 1990, Collection Forum 6: 45-52.