A SIMPLE METHOD FOR SHIPPING FIELD COLLECTED MOSQUITOES FOR ELECTROPHORETIC STUDIES

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ABSTRACT. A method for preserving field collected material in frozen condition for electrophoresis is described. A mixture of ice and salt was used to obtain a freezing temperature of −20°C. Field collected mosquitoes can be stored for up to 9 or more days in an ordinary thermos bottle by replacing the ice-salt mixture at 3-day intervals, without loss of enzyme activity. This method is a considerable improvement for the shipment of material from the field to the laboratory.

During the last 2 decades electrophoresis of isozymes, and more recently DNA, has become a common method for the study of genetic variability in mosquito populations. Isozyme patterns have been used for taxonomic identification of sibling species in a number of anopheline species (Narang and Seawright 1988). Although electrophoretic methods and techniques for histochemical staining systems are relatively easy, one needs either live mosquitoes or material stored at −70°C or lower until used for electrophoresis. This requirement causes a problem, in that portable equipment (e.g., liquid nitrogen or dry ice containers) for freezing mosquitoes must be transported during field collections. It is difficult, and in some cases impossible, to purchase liquid nitrogen, and express mail services often refuse to accept packages containing liquid nitrogen. Many researchers have to hand-carry the frozen material to the laboratory, which adds to travel costs and limits the time available for field collections. Alternatively, the use of dry ice is not practical in the field because of the need for a relatively constant supply, at least at 3-day intervals.

In our cooperative research, we have experienced the inefficiency of abbreviated field trips and the inconvenience of losing samples during shipment. Because of these problems, we have tried other approaches to shipping frozen material. In this paper we describe a simple, inexpensive method using a well-known phenomenon of achievement of very low temperatures by mixing crushed ice with table salt. This temperature can be maintained for 2–3 days which would allow sufficient time for the material to be shipped by an express service.

In laboratory tests to check the feasibility of the salt-ice method for preservation of specimens, we first evaluated the temperatures that could be achieved with various concentrations of salt. The materials needed for preparing mosquitoes for shipment include an ordinary thermos bottle, table salt, crushed ice, a styrofoam mailing box and plastic specimen tubes (Eppendorf tubes). Adult mosquitoes of both sexes were placed into Eppendorf tubes, 10 of which were put in a flask, and covered with the ice-salt mixture, prepared by mixing table salt vigorously with crushed ice. The temperature was checked with a thermometer. The flask was closed, sealed tight with masking tape, and stored at room temperature in a styrofoam box for up to 3 days. Electrophoresis (Steiner and Joslyn 1979) was performed on the mosquitoes removed from the flask. Table 1 lists the temperatures observed for various mixtures of ice and salt. When the 25% (w/w) salt mixture was stored in the thermos bottle, the temperature remained between −20 to −10°C for 48 h, at which point most of the ice had melted. The temperature rose to −2°C after 3 days and 2°C by the 4th day. The samples were tested electrophoretically to determine the stability of allozymes of 18 enzymes as compared to control samples frozen at −70°C. Results indicate that all enzymes were stable for 4 days of storage.

For simulation of field shipment conditions, another flask prepared in the same way was packed in a styrofoam box and mailed by overnight express service to I. C. McDonald at a U.S. Department of Agriculture laboratory in Fargo, North Dakota. The parcel was returned to our laboratory by express mail. All of the specimens retained enzyme activity. This 2-day express-mail experiment simulated the one-way shipping

Table 1. Drop in temperature obtained by mixing table salt and ice in different proportions (5 replications).

% w/w of salt in mixture	Mean temperature°C ± SE
5	−8.2 ± 0.45
10	−14.6 ± 0.55
15	−17.2 ± 0.45
20	−18.8 ± 0.45
25–35	−20.0 ± 0.00

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time usually associated with transport of insects from field to laboratory.

Prolonged storage of specimens was tested by holding 10 tubes (20 mosquitoes/tube) in a vacuum flask, for which the ice-salt mixture was changed at 3-day intervals during a 9-day period. Three tubes were removed from the flask during each change and stored at −70°C until used for electrophoresis. The enzyme activity remained stable throughout the 9-day period.

For practical field application of this method for overseas collections, adult mosquitoes in specimen tubes can be transferred to the bottom of the flask and covered with the salt-ice mixture. After closing it tight, the flask can be shipped in a styrofoam box by an international express mail service. Mosquitoes can then be transferred from the flask to a freezer for storage until needed for electrophoresis or further shipping to the next destination.

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