Freeze-drying Adult Mosquitoes for Taxonomic Study[1]

Ralph E. Harbach and Bruce A. Harrison

Walter Reed Biosystematics Unit
NHB-165, National Museum of Natural History
Washington, D.C. 20560

Abstract. The freeze-drying of adult mosquitoes for taxonomic study was investigated. A procedure was developed that combines the freeze-drying method of preservation with the traditional method of killing and mounting specimens. Specimens prepared by this method are taxonomically more valuable as they show little or no structural distortion, loss of color or loss of scales and setae. Freeze-dried specimens can be used for scanning electron microscopy, and they would make excellent type-material. Other advantages and disadvantages are discussed.

INTRODUCTION

Adult mosquito specimens are distorted considerably when dried under ambient conditions (Fig.1). Anyone who has performed taxonomic studies on mosquitoes knows how difficult, and often frustrating or impossible, it can be to look for anatomical features that are obscured or hidden on collapsed or shrunken specimens.

Two primary methods are available for obtaining undistorted specimens for taxonomic study. These are the acetone-treatment and freeze-drying methods of preservation. Preservation by the acetone-treatment method is not widely known. This is unfortunate, because Truman (1968) developed this simple technique specifically for preserving adult and larval mosquitoes for taxonomic study.

Freeze-drying is a well-known technique that is used extensively to preserve biological specimens for museum display (Hower 1979). The freeze-drying of arthropods was first investigated by Woodring and Blum (1963) who demonstrated that well-preserved specimens of spiders and immature insects

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could be obtained for study as well as display purposes.[2] Numerous species of insects undoubtedly have been freeze-dried for study since Woodring and Blum paved the way, yet information on freeze-drying adult specimens, including mosquitoes, has not appeared in the literature.

Freeze-drying is a technique whereby continuously-frozen specimens are dehydrated by sublimation, under vacuum, without significant loss of physical form or color. Even though perfect specimens are desirable for taxonomic research, we recommend that freeze-dried adults be prepared to complement rather than replace series of normally-dried specimens. We do not regard freeze-drying as a substitute for the acetone-treatment method, but rather as another way of preparing adult mosquitoes for taxonomic study.

METHOD

Because captured live adult mosquitoes are frequently missing scales, setae, appendages or are otherwise damaged, only those specimens obtained through individual, mass or progeny rearings should be freeze-dried for study. Where appropriate, we prefer to freeze-dry 10 females and 10 males of each species collected from rearings. Each reared specimen is anesthetized with ethyl acetate until its legs are extended, and then placed into a petri dish along with a label to identify its associated larval and/or pupal exuvia. The petri dish is held over ice to slow down autolytic enzyme activity that could be destructive to the specimens. After all the specimens have been anesthetized, the petri dish is placed into a freeze-drying apparatus[3] which has been precooled to -30 °C. Rapid freezing is essential, for it results in the formation of smaller ice crystals, causing less damage to membranes and tissues. After the specimens are frozen, they are vacuum-dried for a period of 2-3 days. We routinely dry 20 specimens for 3 days before mounting them on points.

RESULTS AND DISCUSSION

Freeze-drying produces specimens with little or no structural distortion, loss of color or loss of scales and setae (Fig. 2). Minor wrinkling sometimes occurs in membranous areas, probably due to the supercooling of fats and esters, or because of polyphenols in the cuticle (R.O. Hower, pers. comm.). However, those areas which usually become distorted during normal drying, e.g., the femora, proboscis, compound eyes, thoracic pleura and abdominal sterna, are always well preserved. The compound eyes invariably assume a bleached appearance, presumably because of ice forming on the outsides of cells, causing the internal tissues to separate from the cuticle (R.O. Hower,

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[2] In the same year, these authors (Blum and Woodring 1963) also reported on the preservation of insect larvae by vacuum dehydration, a modification of freeze-drying whereby specimens are frozen, then dried in a vacuum chamber held at room temperature.

[3] Freeze-driers are commercially available in a variety of sizes and styles to meet individual needs. A VIRTIS model 10-PR, manufactured by The Virtis Co., Inc., Gardner, N.Y. 12525, was employed in our studies.

pers. comm.). But the eyes do not collapse as usual, and thus the structural integrity of the head is maintained.

Freeze-dried specimens retain their natural color because pigments are frozen in place. Even the delicate green tint in teneral adults of certain species is preserved. This, however, may not be true of specimens treated with acetone, because coloration due to soluble pigments may be altered or lost in tissues treated with this solvent (R.O. Hower, pers. comm.). Structural color is not affected by either method.

Besides being valuable for classical taxonomic research, freeze-dried specimens are suitable for examination in the scanning electron microscope (SEM). Specimens only need to be properly mounted and coated with a heavy metal, and they are ready for study. Unfortunately, observations on fine structure and three-dimensionality are made at the expense of those characters which are obliterated during coating. For this reason, specimens should be prepared for SEM studies only after they have been examined thoroughly and information on color and other characters has been recorded.

Taxonomists must often rely on badly distorted type-specimens to establish species identities. Clearly, freeze-dried specimens would make excellent type-material. In addition, it would be advantageous to freeze-dry topotypic material for study.

The major disadvantage to freeze-drying is that it requires an expensive apparatus which is not always available to researchers. Another drawback is that the procedure requires several days to complete. By contrast, preservation by the acetone-treatment method is accomplished in about 3 hours with inexpensive materials that are ordinarily found in the laboratory. On the other hand, freeze-drying is safe, while acetone vapors are harmful to breathe.

Although it would be impossible for collectors to freeze-dry specimens in the field, under certain circumstances, material could be frozen and transported back to the laboratory. The danger here is that frozen specimens may slowly shrink and deteriorate. Blum and Woodring (1963) found that the larvae of many lepidopterous species are distorted by superficial drying after being continuously frozen for more than a few weeks.

Despite limitations, freeze-drying is a satisfactory method of preparing specimens for taxonomic study. Adult mosquitoes preserved by this method require no special care, and are no more delicate than specimens dried in the usual manner. To date, we have freeze-dried adults of the following species with excellent results: *Culex quinquefasciatus* Say, *Cx. pipiens* L., *Cx. molestus* Forskal, *Aedes aegypti* (L.) and *Anopheles stephensi* Liston. Ideally, each collection of individually-reared material should contain a series of freeze-dried adult specimens.

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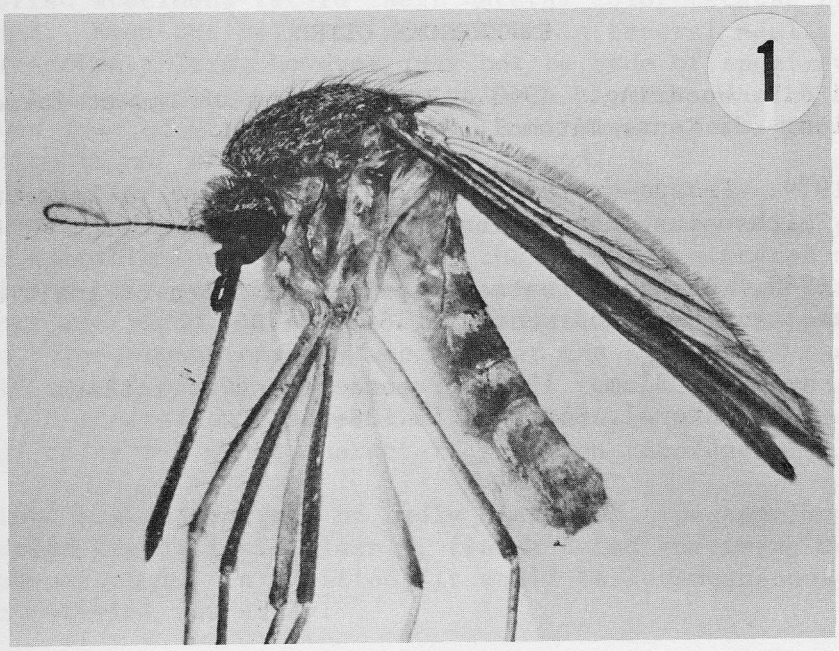

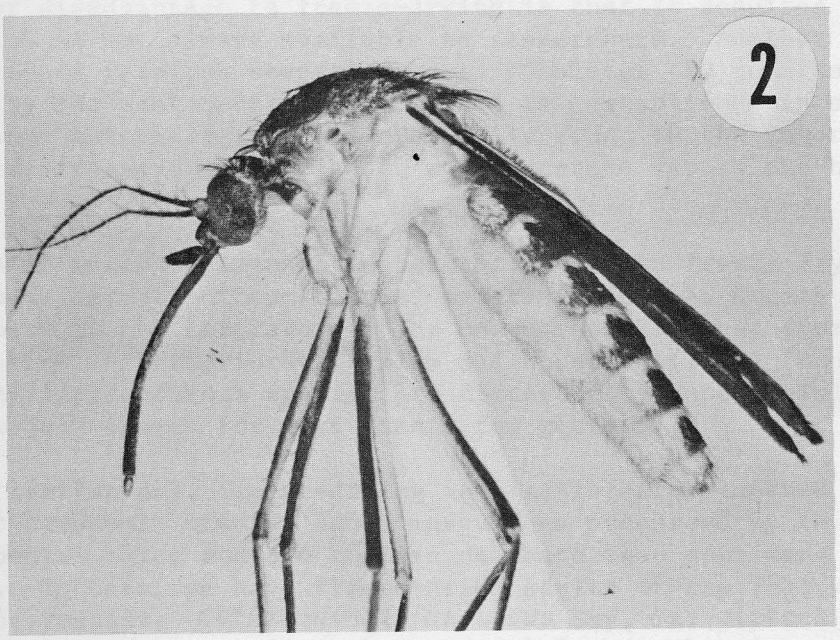

Figs. 1,2. Individually-reared females of *Culex (Culex) quinquefasciatus* Say from Johannesburg, South Africa.

1. Dried under ambient conditions.
2. Freeze-dried.