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Freeze-drying : Entomological Applications

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Summary

Some fresh or ethanol preserved soft bodied Arthropods have been tested to freeze-drying. Amongst the tested material, general shape and volume as well as natural colors have been preserved. Freeze-drying may have some interesting applications in the creation of entomological reference collections of dry specimens (including preimaginal stages) and in electron microscopy.

Keywords : entomological techniques, preservation of dried specimens, freeze-drying.

Résumé

Quelques Arthropodes à corps mou, frais ou conservés dans de l'éthanol, ont été lyophilisés. Parmi le matériel essayé, la forme générale et le volume aussi bien que les couleurs naturelles ont été préservées. Cette technique pourrait trouver d'intéressantes applications dans la création de collections entomologiques de référence de matériel conservé à sec (y incluant les états préimaginaux) et dans le domaine de la microscopie électronique.

Introduction

The method of freeze-drying is far from being a new technique since its principles date back to 1906. In the case that interests us, it consists of the volatilization, at low temperature, of the ice contained in the tissues of an organism. Its principle rests on a physical phenomenon known as sublimation, where a solid material passes directly to a gaseous state, without passing through a liquid phase. In order to achieve this, and briefly stated, one has first to freeze completely, but also quickly, the body; then, secondly, to place the body in a container where a vacuum is created. It is this lowering of pressure on one side, and a low warming of the body (without reaching water's melting point however) on the other side, that will allow sublimation of frozen water contained in the tissues. One of the big advantages of this method, and unlike the other ways of drying, is that the original volume as well as the general shape of the body remain unchanged.

Freeze-drying was first used for the preservation of bacteria, serums and toxins around 1935 in the USA. Its first industrial applications, in 1940, was for the conservation of dried human blood plasma : a technique that saved many lives (DORVAULT, 1978; ROMOND, 1980). Since that time, and despite its relatively high cost that prevent it from being even more widely applied, this method has found numerous uses in many areas, including in the daily life of millions of people : in pharmaceutical industry : serums, vaccines, blood, antibiotics, etc.; in microbiology : viruses, bacteria, yeasts; veterinary medicine : preservation of blood, sperm, medicines; but the most popular freeze-dried products are those occurring in foodstuffs : coffee, cocoa, milk, vegetables and spices (" quick " soups, spice cubes), etc.

Although the author has been aware of the existence of this technique for some two decades and has always thought that it could find some interesting applications in entomology, opportunities to test it never occurred until recently, when wishing to examine larvae of donaciines by means of scanning electron microscope I looked for a way of drying specimens without changing

Fig. 1. *Isotoma* sp. (Collembola Entomobryidae), lateral view. Freeze-dried ethanol preserved specimen.

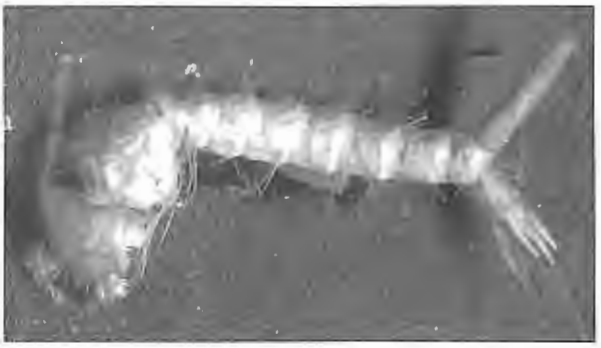

Fig. 2. Last instar larva of *Culex pipiens* L. (Diptera Culicidae), dorso-lateral view. Freeze-dried ethanol preserved specimen.

Fig. 3. *Enoplognatha ovala* (CLERK) (Araneae, Theridiidae), lateral view. Freeze-dried fresh specimen.

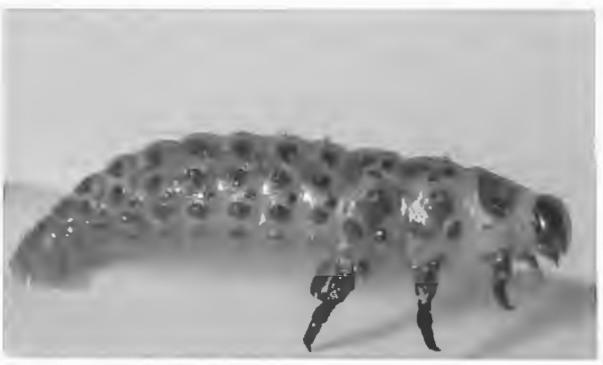

Fig. 4. Last instar larva of *Chrysomela vigintipunctata* (SCOPOLI) (Coleoptera Chrysomelidae Chrysomelinae), lateral view. Freeze-dried fresh specimen.

Fig. 5. Last instar larva of *Chrysomela vigintipunctata* (SCOPOLI) (Coleoptera Chrysomelidae Chrysomelinae), dorsal view. Freeze-dried fresh specimen.

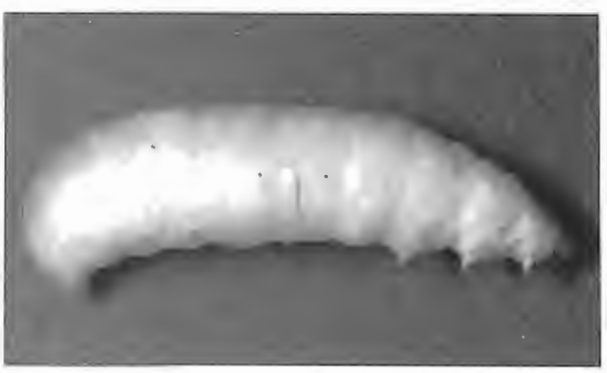

Fig. 6. Last instar larva of *Plateumaris sericea* L. (Coleoptera Chrysomelidae Donaciinae). Freeze-dried ethanol preserved specimen.

Fig. 7. Pupa of *Chrysomela vigintipunctata* (SCOPOLI) (Coleoptera Chrysomelidae Chrysomelinae), ventral view. Freeze-dried fresh specimen.

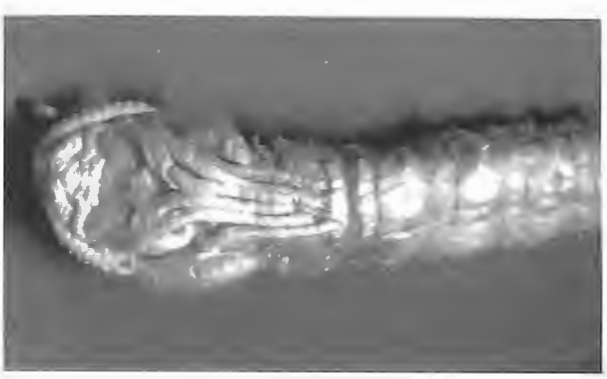

Fig. 8. Pupa of Tipulidae (Diptera), ventral view. Freeze-dried ethanol preserved specimen.

their volume, and then thought back to the freeze-drying method. In a recent article I suggested (LAYS, 2001) that this technique could find some entomological applications, which is now confirmed.

Applications

Two kinds of specimens have been tested : fresh material, collected a few days before testing and killed just a few hours earlier with vapours of ethyl acetate; and 70° ethanol preserved material (some of them being in alcohol for some months, some others for years). Since the freeze-drying device contains rubber joints that can not enter into contact with ethanol vapours, or any other organic solvents, it is necessary to submerge, during several days (3 in this case) the ethanol preserved specimens in a large volume of distilled water, in order to replace the former by the latter. After that, specimens are placed in glass tubes. Usually specimens do not need to be submerged in distilled water when placed in those tubes, but sometimes that can be necessary. In fact frozen water of small specimens (or part of them), during the times of handling (when transferred from liquid nitrogen to freeze-dryer) starts to thaw and as a consequence, when a vacuum is created some parts of internal structures may come out (*e.g.* : intestines of fresh spiders coming out of the abdomen); in order to avoid this, specimens will remain immersed in a small quantity of distilled water before being transferred to liquid nitrogen. Top of tubes are covered with a thin film of paraffin (Parafilm M) which is perforated sereral times with a small needle; the film prevents specimens from getting out of the tubes and holes allow sublimation to take place. The tubes are then placed (halfway down) in liquid nitrogen (-196°C) for 10 minutes, with specimens placed on the tube's bottom. The tubes are then enclosed in a Virtis freeze-drying apparatus for 16 hours under a pressure of 11 millitorr and at a temperature of - 53°C.

Different kinds of specimens have been tested with very satisfactory results, as can be noted on the photographs (Figs 1-8). Aside from the specimens shown here, two ethanol preserved specimens were also tested : one of a crab spider (Thomisidae) and one of a chafer larva, both gave good results.

The natural colors of pupae and larvae of *Chrysomela vigintipunctata* were maintained (Figs 4, 5, 7); to a lesser degree, this is also true for the small spider *Enoplognatha ovala* (Fig. 3), but it should be possible to ameliorate the technique in order to preserve specimens' natural colors. That could be then interesting for the dry collections of, for instance, caterpillars. On some specimens that were preserved in ethanol, small quantities of whitish and amorphous substances occurred (that could be some salts), they can be easily removed with a small bristle impregnated with ethanol or methanol.

Theorically, any organism can be freeze-dried (flowers, slugs, tadpoles, caterpillars, etc.). Although the technique is ancient, its applications in natural history collections could be probably more widespread. For entomological collections for instance, one could imagine the creation of reference collections where all stages of one species, from ova to imago, are gathered on only a few pins, instead of having these stages preserved by several means and scattered in different places.

Aside from these preservation purposes, the technique may find interesting applications when one wishes to examine material by scanning electron microscope. Material preserved in ethanol or fresh material must be first dried before any examination with a S.E.M. Under classical means of drying (air, heat, oven, etc.) specimens shrivel up, creating folds that hide structures. With the freeze-drying technique, that keeps intact the original shape and volume, the problem is solved.

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