# A SIMPLIFIED METHOD OF FREEZE-DRYING CATERPILLARS

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In recent years freeze-drying of museum specimens has been studied and has provided means of obtaining specimens up to the size of a hamster in their natural form without too much difficulty (Meryman 1960, 1961). Application of this method to insects, particularly to caterpillars, has yielded satisfactory results (Blum and Woodring, 1963; Woodring and Blum, 1963). However, the vacuum or pump systems (Meryman, 1959) employed in the methods, while not overly troublesome for a laboratory, are generally more than an average collector can afford. For caterpillars and other small specimens a vacuum or a pump, while desirable for speeding the process, is not a necessary requirement for the same result, as is shown in the present paper.

Freeze-drying is based on the fact that ice evaporates just as water does, although at a much slower rate. In more scientific terms, the "partial pressure of water vapor" over ice is quite low. That means that if ice is brought into a dry atmosphere in a closed system at low temperature, only a small amount of ice needs to evaporate in order to saturate the atmosphere with water vapor. Once this state of equilibrium is reached no more ice evaporates. If the water vapor is removed, the equilibrium is disturbed and is restored by further evaporation of ice. The removal of the water vapor can be achieved, for example, by passing a stream of dry, that is, water-free air over the ice; or, as in the method to be presented, by binding the vapor on an appropriate agent, called a desiccant. Of course, the whole process must be done at a low temperature in order to prevent melting of the ice. Operation under low pressure, that is, in an evacuated system, has the advantage that the water molecules can readily move away from the surface of the ice and when using a desiccant rapidly reach its surface where they are bound. However, the principal process of water evaporating from the ice and moving to the desiccant takes place under normal pressure too, although more slowly. With respect to time it would seem best to operate at as high a temperature as possible but of course without exceeding that of the melting point. The transfer of the water vapor from the surface of the specimen to the desiccant is not the only process to be considered. Water vapor from within the specimen has also to penetrate the integument in order to reach the surface. This process too is accelerated by increasing the temperature. In addition, however, higher temperature also speeds the decay of the specimen. In order to

avoid the latter, a temperature below the optimum for speedy drying must be maintained. Such considerations are of mere theoretical interest since the temperature in the ice cube compartment is preset and in the freezer variations are possible only within a limited range for the collector working at home.

With this admittedly sketchy discussion of the underlying principle it will be easily understood how freeze-drying of a caterpillar is effected. The animal is brought into a container together with some desiccant. The container is then closed air-tight, stored in a deep freezer or in the ice cube compartment of a refrigerator and left there until essentially all the water from the frozen specimen has moved to the desiccant. The time required for this process predominantly depends on the size of the specimen and the temperature (see above). There is no formula established to allow an exact calculation or even a rough estimation of this time, and each experimentor will have to gain his own experience. But some idea may be obtained from the series of experiments described below.

The containers present no problem. Any tightly closing screw cap glass or plastic container is suitable provided its size fits the dimensions of the specimen.

Desiccants suitable for the purpose exist in large numbers. We consider Silicagel as the most practical one. It is readily obtained at a low price from a supply house for chemicals. Silicagel is sold as granules of 1–2 mm diameter and in dry state is of deep blue color. This color is due to an impregnation with a salt that changes its color to pink when wet. Thus the color of the material readily indicates exhaustion. The pink Silicagel no longer has water-absorbing properties and consequently the charge in the container must be renewed. Silicagel has another enormous advantage, namely, it can readily be regenerated by mere warming. A collector who has no access to special drying ovens can perform this task by simply placing the pink Silicagel into a pot or frying pan and keeping it at moderate heat, while occasionally stirring it with a piece of wood or a spoon. The heating may be effected on the burner of a range or, preferably, because it is milder and more uniform, in an oven. No harmful components are released. When the mass has completely regained the blue color, it is placed back into the storage bottle, kept there tightly closed and is ready for reuse. Extreme heat should be avoided during the drying procedure because it may cause cracking of the particles and render them less effective in their drying power. As a rule of thumb the lowest temperature that achieves blueing should be used.

During experimentation over more than two seasons some experience has been acquired, and a few points of interest may be discussed, but

anyone applying the method may find ways of improvement in one respect or another. A caterpillar that is sluggish and does not move around, *e.g.*, a saddle-back caterpillar, can be brought with the leaf on which it sits directly into the desiccant-containing jar, which after closing is placed directly into the freezer. The animal dies from undercooling with hardly any movement and the drying process starts to take place.

Species that move about can also be treated in this way but in many cases will curl or contract before they die and will then not show their natural position or form. In such an event it is often helpful to put the caterpillar in a container without desiccant and to place it in a refrigerator (not freezer!). At the reduced temperature the animal commonly ceases moving and while still alive comes to rest in a natural position. Once this state is reached the container is transferred to the deep frost compartment or freezer where the caterpillar solidifies in the desired position. When thoroughly frozen (say, overnight) the specimen is transferred to the desiccant-containing jar. It is advisable to make this transfer as rapidly as possible in order to prevent large amounts of moisture from condensing on the specimen. This condensed water, of course, must also be removed and more drying time is required and more desiccant is used up. In this modification of the method it is also advisable to have the desiccant-containing jar cooled before introducing the specimen. The reason for this is that the outside portion of the specimen in contact with the desiccants may thaw sufficiently to become flat or get dells impressed from the granules of the Silicagel. Of course such distortions once the specimen is dried cannot be repaired.

The final method is to kill the caterpillar before subjecting it to freeze-drying by dropping it into boiling water. After removal from the water it is essential to place the specimen on a blotting paper and to allow evaporation of the adherent water. Hairy specimens after such treatment show the hairs completely entangled. But natural position of the hairs can readily be restored by brushing with a soft brush after complete outside dryness has been reached. In order to avoid dells the specimen should not be brought directly into the Silicagel but rather be placed on a piece of paper or thin cardboard. After arranging the specimen in the desired position, the jar is closed and carefully placed into the freezer.

It is advisable to inspect the jars in the freezer from time to time and observe the progressive expansion of the pink layer in the Silicagel. If its major portion is pink the desiccant should be renewed. In order to avoid thawing of the specimen, the following procedure is recommended. A jar with new desiccant is placed into the freezer and allowed to cool. Then this jar and the one containing the specimen and the exhausted

desiccant are removed from the freezer, both rapidly opened, and the specimen is quickly (to avoid condensation of water) transferred to the new jar; the jar is closed at once and returned to the freezer.

Renewal of desiccant is rarely necessary. The occasion usually only occurs when an extremely large specimen was placed in an unappropriately small container, or too many specimens were put into one jar, or too little desiccant was added. Some experiences will soon establish the minimum amount of desiccant necessary to completely avoid renewal of the charge. During the study most cases where renewal became necessary arose due to a cap that did not fit tight enough and thus permitted entrance of humidity from without.

A frost-free freezer is not a necessity but offers a great advantage. It allows inspection of the contents of jars without hindrance by ice accumulation on the outer walls.

In all cases studied by the authors the form of the specimen was retained perfectly. This held for specimens in sizes from that of a small skipper larva to the caterpillar of *Citheronia regalis* (Fabricius) and included hairy specimens like *Syntomeida epilais jucundissima* Dyar. Unfortunately, the situation with respect to the retention of color is not as favorable. Some colors are kept, others are not. The discoloration usually does not take place during the drying itself but is a slow process that occurs during the later storage of the dried specimen. It seems that the color green especially tends to fade. However, this cannot be ascribed to the freeze-drying method as such but is rather due to the particular chemical behavior of chlorophyll, the pigment usually responsible for this green color. Thus, description of the color on a label accompanying the specimen or a color photograph is recommended for permanent records. Some caterpillars show a shine in natural conditions and this may be lost during the freeze-drying. With non-hairy specimens the shine can be restored by spraying with a transparent lacquer. In order to avoid attacks by various museum pests it is advisable to spray the finally dried specimen with one of the commercial household or garden insecticides (Hess bomb, Raid, etc.).

The following data will give a rough idea about the quantitative aspects of the process. The experiments were carried out in the following manner. Caterpillars of *Malacosoma americana* (Fabricius) were weighed and put into small screw-cap plastic vials containing an adequate charge of Silicagel. The containers were then placed into the ice cube compartment of a refrigerator. Jars were taken after a specified number of days and allowed to warm to room temperature. The specimens were then removed from the jars and weighed again. These weights as well as the original

weights and weight losses in grams and also in per cent are tabulated in Table I. Specimen No. 3, after being removed from the jar and weighed, was placed into an oven and dried at 110°C to constant weight (overnight). The total weight loss, that is, referred to the original weight amounted to 82.5%. A fresh specimen of 0.631 grams was killed and immediately dried in an oven at the same temperature and it showed a total weight loss of 82.7%. These data were used to obtain the figures in the last column expressing the weight loss with respect to moisture. It must, of course, be realized that this figure represents not only loss of water but includes that of some other volatilizable material.

Inspection of the data in Table I shows that freeze-drying beyond a certain time does no longer produce a significant removal of moisture. After 550 days only 94% water is removed. This might be taken as an indication of incomplete drying. However, caution must be exercised in such a judgment. It is highly probable that complete dryness (that is, removal of moisture) has been attained and that the remaining 6% are partly nonaqueous volatile materials and chemically bound water. It is enough to reduce the water content of the specimen to a degree sufficient to prevent decay and this seems to be achieved with a water content of only about 10%. Specimen No. 3 after having been freeze-dried to a loss of about 50% and then for final, rapid drying, was placed into an oven. No decay or other adverse phenomena were observed. From this experiment the conclusion may be drawn to a possible short-cut of the methods. It seems to be sufficient to freeze-dry the specimens to a loss of about 40–50% of their moisture and then to dry them to completion at an elevated temperature. It may also be possible to speed the process by removing the jars after about 40% drying from the freezer and then allow to stand at room temperature where faster final drying can be achieved. However, this will require further investigation and at the present time

TABLE I. DATA ON FREEZE-DRYING LARVAE OF *Malacosoma americana* (FABRICIUS).

| Specimen Number | Days | Original weight in grams | Weight in grams after specified days | Weight loss in grams | Weight loss in % | % Loss expressed as moisture |
|---|---|---|---|---|---|---|
| 1 | 1 | 0.386 | 0.381 | 0.005 | 1.3 | 1.6 |
| 2 | 10 | 0.459 | 0.438 | 0.021 | 4.7 | 5.7 |
| 3 | 40 | 0.699 | 0.405 | 0.294 | 42 | 51 |
| 4 | 100 | 0.481 | 0.137 | 0.344 | 72 | 87 |
| 5 | 111 | 0.417 | 0.103 | 0.314 | 75 | 91 |
| 6 | 550 | 0.669 | 0.151 | 0.518 | 77.5 | 94 |

the simplest and safest way is to place the specimen into the desiccant-containing jar and allow to stand in the freezer for about 3–6 months depending on the size of the caterpillar. The well-known tent caterpillar *Malacosoma americana* should readily serve as a guide. The specimens used in the experiments were in their last instar and about one inch in length.

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## A NEW FOODPLANT FOR *EUPHYDRYAS PHAETON* (NYMPHALIDAE)

On July 17, 1967, a wet meadow near Newton, New Jersey, was visited where *Melitaea harrisii* Scudder and *Euphydryas phaeton* (Drury) were plentiful. Whenever I find *E. phaeton* flying, I look them over first for variations, then try to locate the foodplant, turtlehead (*Chelone glabra* Linnaeus), to find the larva of *Papaipema nepheleptena* Dyar (Noctuidae) boring in the root of this plant. This meadow and several others in the neighborhood were investigated thoroughly, but no turtlehead could be found. Later, a larva of *E. phaeton* was found on a plant with white flowers, and further investigation enabled collection of a dozen larvae of all sizes in the space of 15 minutes. The caterpillars were sitting on the top of either leaves or flowers where they could be easily detected. This plant, which was growing in this meadow by the hundreds, was identified as eastern pentstemon (*Pentstemon hirsutus* Linnaeus), or hairy beard-tongue. It grows from one to three feet high, with the flowers one inch wide. The range of this plant is said to be the eastern half of the U.S.A. and adjacent Canada. Like turtlehead, it belongs to the snapdragon family, Scrophulariaceae.

Turtlehead being extremely rare in the New Jersey area, larvae of *E. phaeton* should therefore be expected on pentstemon.

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