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Some notes on freeze drying spiders

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Summary

The process of freeze drying spiders for collections and exhibits is described. The physical and chemical changes brought about by this process are outlined since it is necessary to understand them in order to overcome problems that may arise during freeze drying. The limitations of the process are also described and a series of photographs is included to illustrate spiders and their genitalia preserved by this method.

Introduction

Freeze drying is a controlled process for removing water from the cells of biological specimens leaving the tissues dry, rigid and exactly preserved (see Harris, 1964). The frozen specimen is processed below freezing point under high vacuum, so that ice crystals in the tissues are gradually sublimed into water vapour and are thus removed. Certain compounds, such as lipids, which are not miscible with water, and freeze-resistant substances such as glycerol, are often present in the body and may prevent it from drying. However, exposure to a very low temperature (below $-100^{\circ}C$), can induce a chemical breakdown of these viscous substances into simpler molecules which overcomes the problem. The process usually produces a spider with its natural colouration (Plates 1-5), body shape and important structures such as genitalia and eyes exactly preserved (Plates 6-10). Although very low temperatures will often affect cutaneous colour, pigmented hairs on spiders' bodies remain unchanged. A spider preserved by freeze drying will therefore be useful in museum exhibits and dioramas, having the advantages of better colour preservation than liquid preserving agents could achieve, no distortion of the body and easier handling facilities.

Technique

Spiders that are not colourful, (e.g. darkly coloured mygales), may be fixed in alcohol if the specimen dies prematurely, but alcohol must be thoroughly washed out of the specimen with tap and then deionised water before freezing.

1. Kill the spider in ethyl acetate vapour and pin it out as desired on balsa wood (cork tends to warp at low temperature).
2. Place spider into refrigerator for 1 hour to allow muscular contractions to cease.
3. If any limbs have moved, reset them. Freeze specimen at $-20^{\circ}C$ for 3 days. Prolonged undisturbed freezing is helpful and allows the more viscous body fluids to be slightly denatured which helps freeze drying. The presence of mature eggs in a female raises the level of viscous fluids and sometimes causes the abdomen to reject freezing and dark spots appear on the skin surface.
4. Check that the abdomen is frozen hard; if at all flaccid, use polar spray (aerosol), until a white frost is noticeable. This may lighten natural colours.
5. Weigh the specimen and place into freeze dryer for approximately 48 hours; reweigh and continue

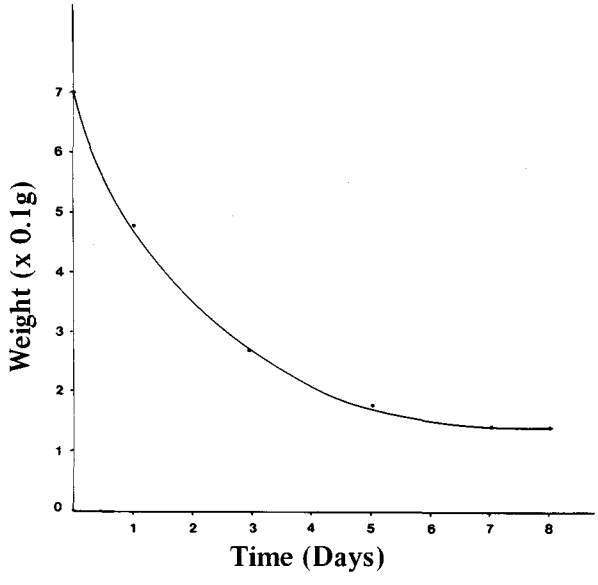


Fig. 1: Freeze-drying curve of a semi-gravid female *Araneus diadematus* Clerck, constant body weight achieved after 8 days and 80% weight loss (initial frozen weight 0.78g, final dry weight 0.148g).

process until constant body weight is achieved (Fig. 1). The time taken to achieve constant body weight varies according to the size of the specimen and the viscosity of its body fluids. An average spider, e.g. *Tegenaria, Araneus,* will take between 5 and 8 days if no problems are encountered. Freeze drying is carried out at −5 to −10 °C and at a vacuum decreasing to below 0.1 Torr (Moore, 1976).

6. Allow the specimen to achieve room temperature in the presence of a desiccant. Condensation from cold metal objects such as the setting pins may minutely rehydrate the specimen and bring about progressive deterioration.
7. Remove pins etc. and examine the spider carefully to check its state of preservation. Hairs, spines and trichobothria can be damaged through careless handling.

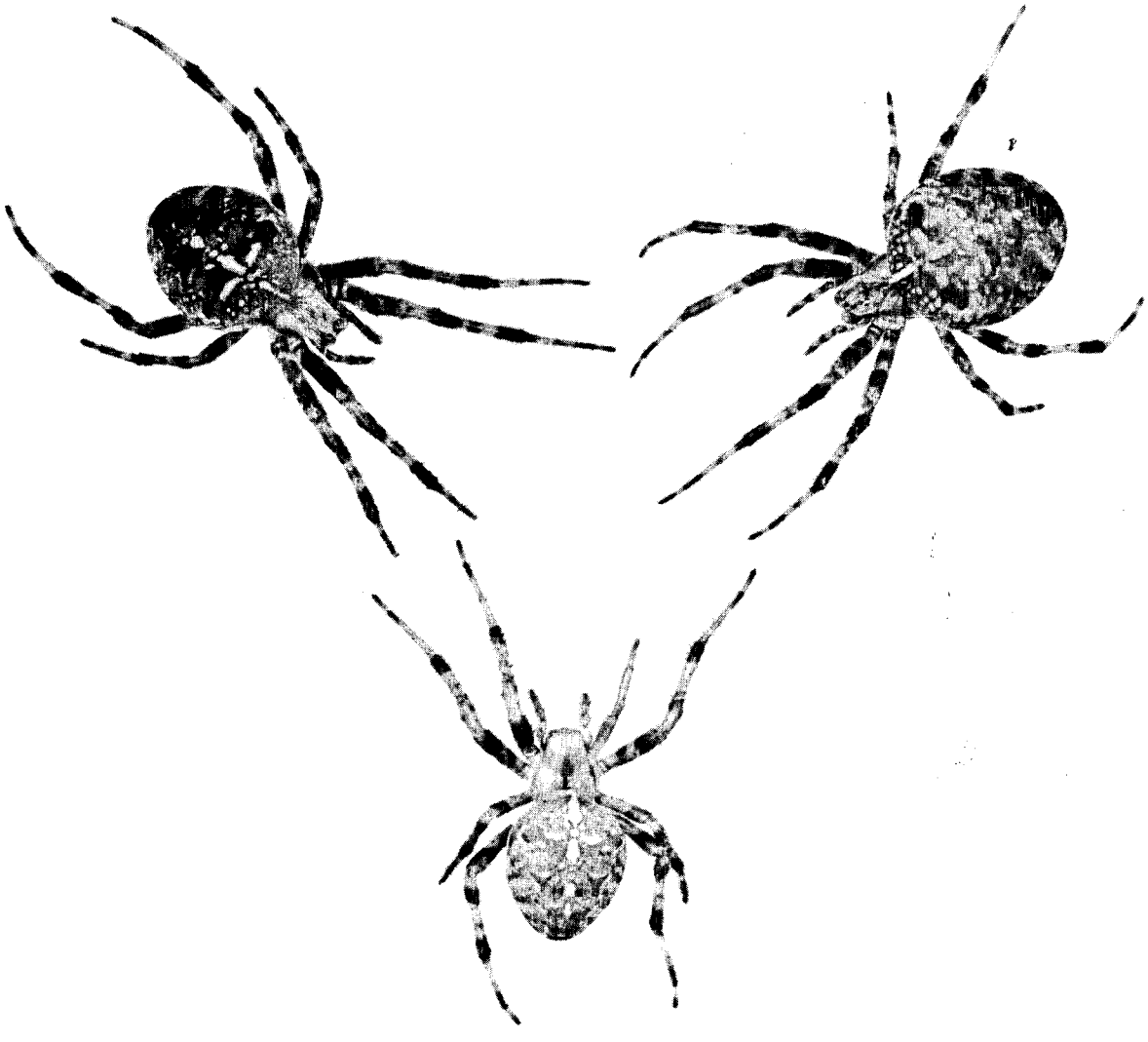
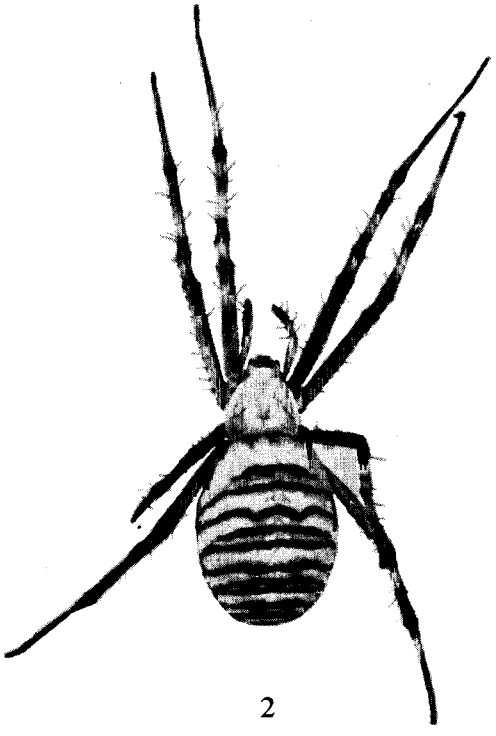


Plate 1: Three freeze dried *Araneus diadematus* showing different colourations (pale brown to nearly black). The palest spider has partially regenerated its right leg I. (x 2.7).



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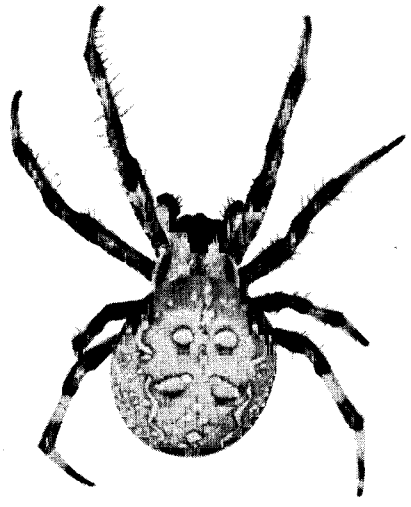

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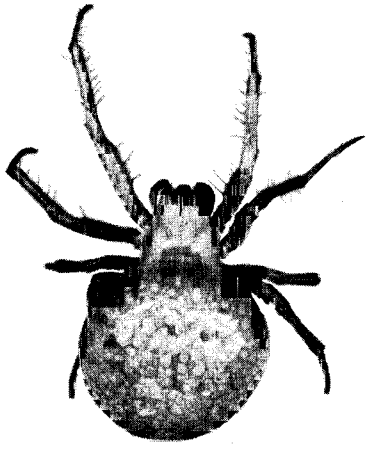

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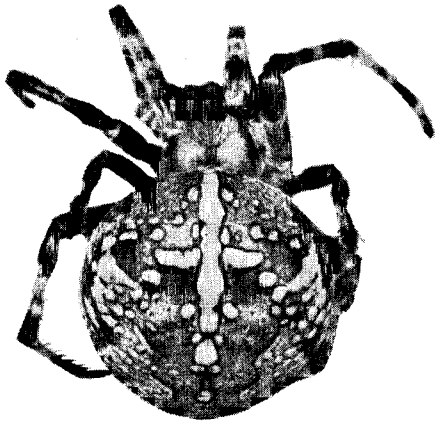

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Plates 2-5: 2 *Argiope bruennichi* (Scopoli), freeze dried to constant weight, 8 days (processed over 7 years ago); 3 *Araneus quadratus* Clerck, freeze dried to constant weight, 10 days (longer than usual due to semi-gravidity); 4 *Araneus alsine* (Walckenaer), freeze dried to constant weight, 5 days (processed over 2 years ago, still retains its bright red colour); 5 *Araneus diadematus,* freeze dried to constant weight, 12 days (abdominal pattern surprisingly well preserved as this was a heavily gravid specimen, processed over 7 years ago. (All x 2.8).

How to deal with problems

Fig. 1 shows that freeze drying is predictably rapid at first. If a noticeable percentage weight loss is not achieved within 48 hours of freeze drying, the specimen may be resisting the process. If the spider contains a high proportion of viscous fluids they will be retained, but lower concentrations will sublime out in the water vapour. If the limbs are not rigid or the abdomen is flaccid, the spider will not be freeze-dried and it will have probably started to decompose, even at $-10^{\circ}C$. Furthermore, natural colouration will probably have been ruined and if this is important, a fresh specimen will have to be obtained. If not, the spider can be quickly refrozen with polar spray until white with frost. A few cm^3 of liquid nitrogen are then carefully sprinkled around the body. The extreme low temperature will denature the viscous body fluids enough for the spider to be properly freeze-dried. The nitrogen must not be poured over the specimen, as this drastic treatment will eventually give rise to splits in the delicate abdominal skin. Sometimes the viscosity of the spider's body fluids will cause the abdomen to decompose in the freeze dryer so that the abdomen collapses. However it can be reinflated by a ventral injection of deionised water and immediate freezing with polar spray. When frozen, the needle is withdrawn and the specimen resprayed before placing in the freezer. This requires some handling experience of the strength of the abdominal skin. On average about 30% of freeze-dried spiders have required some intermediate treatment.

Plates 1-8 illustrate some spiders that have been processed in the museum preservation laboratory, to show as precisely as possible the actual state of preservation achieved by freeze drying. The palpus and epigynal scape (Plates 9-10) have been enlarged by using the Scanning Electron Microscope to show the perfection of their preservation and to show that freeze drying is useful for preparing external as well as internal organs for stereoscan examination (Moore, 1976).

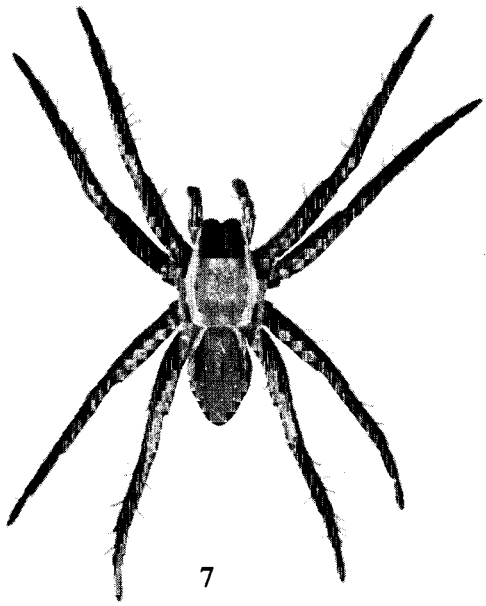
Storage

Freeze-dried spiders should be stored like entomological specimens. If they are to be mounted on card pegs, care must be taken not to cover important areas (e.g. epigyne). To maintain the colour preservation, spiders should be kept in the dark. The internal organs will have been perfectly preserved and may be examined by dry dissection. If desired, the specimen may be rehydrated by immersion in a warm 1% solution of trisodium phosphate ($Na_3PO_4.12H_2O$) for up to 30 minutes. Such a process will impair natural colouration drastically.

Limitations

The cost of freeze drying equipment is high, the machines used to prepare the specimens illustrated in Plates 1-10 costing in the region of £2,500 (see Harris, 1968); however, many hospitals, university departments and other technical institutions have freeze-drying facilities. Apart from the viscosity problem, natural colouration is usually not quite as good as in the living specimen and dermal colours are often slightly faded by the process. The evasive translucent green colour of *Micrommata virescens* (Clerck), is

Plate 6: *Dolomedes fimbriatus* (Clerck) face, clearly showing six eyes, the other two being in profile under the tufts of hair (x 17).



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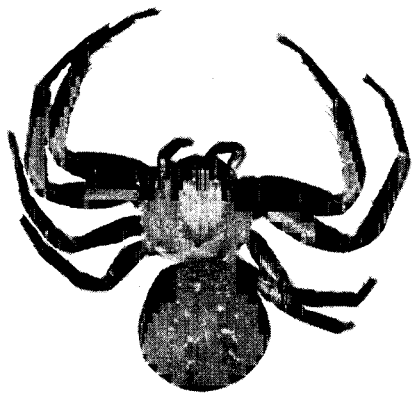

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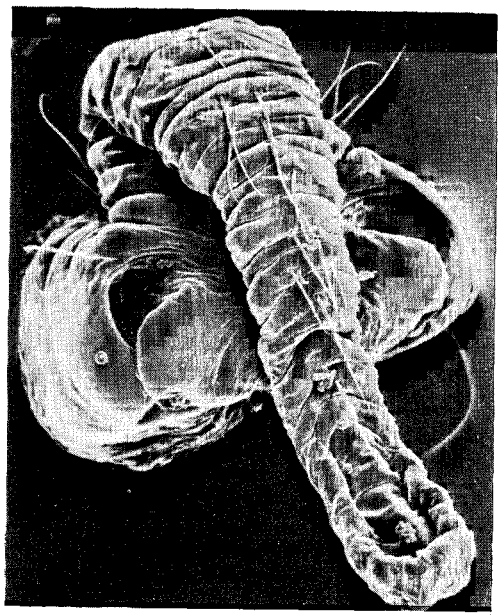

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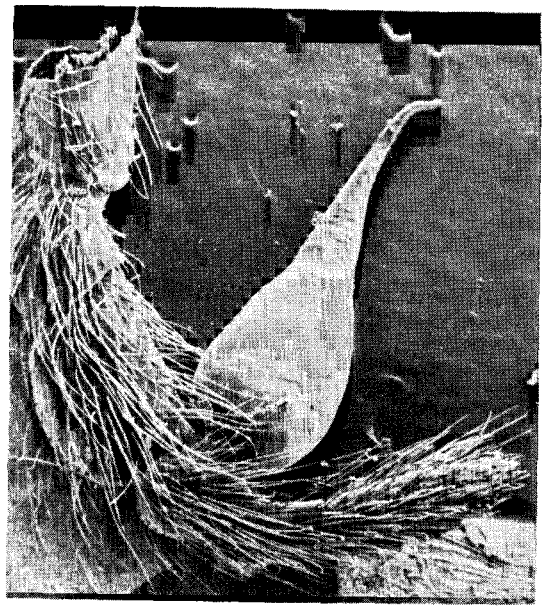

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Plates 7-10: **7** *Dolomedes fimbriatus*, freeze dried to constant weight, 5 days (face shown in Plate 6) (x 3); **8** *Xysticus luctuosus* (Blackwall), freeze dried to constant weight, 5 days (x 3); **9** Stereoscan electron photomicrograph showing epigyne and scape of *Araneus diadematus* well preserved (18 months after freeze drying) (x 150); **10** Stereoscan electron photomicrograph, male palpus of *Segestria florentina* (Rossi) (x 55).

unfortunately lost. The spider becomes opaque as soon as the ice-vapour is sublimed out and the green colour fades quickly. The best result I have achieved with this spider was an initial very pale green colour; 18 months later there was only a hint of green remaining but in another year it will be pale brown.

Coloured hairs as found on the male *Eresus niger* (Petagna), are invariably unchanged in colour and should remain so indefinitely if stored in the dark. Freeze-dried spiders are extremely fragile – especially pholcids whose limbs can fall off if breathed on. With a little skill they can be reattached to the cephalothorax with clear cement.

References

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