## PREPARING LEPIDOPTERA FOR CLASS STUDY

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Of the major orders of insects the Lepidoptera are probably the least adequately covered in most introductory courses in entomology. Students are inclined to place more reliance on general appearance and color patterns than to structural characters and to compare specimens with numerous published figures rather than determine them with the aid of keys. Instructors often encourage this attitude by restricting the material to a few conspicuous species. At the same time the phenomena of mimicry and other types of adaptive coloration are usually illustrated with butterflies and moths.

This situation is largely due to the fact that the identification of the families of Lepidoptera is dependent to a large extent on the examination of details of wing venation which are obscured by the coloration of the scales. To circumvent this difficulty in observation two methods have been used in the past. One involves removing wings, bleaching them, and mounting on slides, a very laborious and time-consuming method of preparation by the instructor. The other consists of temporarily clearing the area of the wing to be observed by the application of a small quantity of alcohol or benzene by means of a fine brush, an exasperating procedure for the student. Both methods result in a very superficial treatment of this important order of insects.



Fig. 1. Spread butterfly with right side bleached to show venation

Recently we have used successfully a simple method of preparing butterflies and moths for student identification. This method to our knowledge has not been used before, probably because most entomologists are loathe to "ruin" a spread lepidopteron by disturbing in any way its scale pattern. Essentially the procedure is as follows:

1. The right fore and hind wings of a pinned and spread specimen are dipped into 95% alcohol in a sufficiently deep container to wet the scales, veins and membrane. The subsequent bleaching will extend only as far

as the wings have been wetted. The specimen should not be left in the alcohol too long as there is a tendency for the long scales of the thorax to draw up the alcohol.

2. The wetted wings are immediately dipped into full strength Clorox (a commercial preparation containing approximately 5.25% sodium hypochlorite in water) or a similar aqueous solution of a hypochlorite such as photographic hypo. The wings are left in the Clorox until a sufficient degree of bleaching has been obtained. This is usually about one or two minutes, but is less with very delicate forms and considerably more for very heavily pigmented and thick winged forms. It is not advisable to leave the specimen more than a few minutes in the aqueous solution, for the wing may become softened. Therefore we prefer to use a strong solution for a shorter period. It is also preferable not to bleach the specimen too much, for it becomes considerably lighter after it dries.

3. After the desired bleaching has been obtained, the cleared wings are transferred to another container of 95% alcohol for a few seconds to wash off the excess bleach and to dehydrate and stiffen the wings. The wash alcohol should be changed frequently to be effective.

4. The specimen is removed from the wash alcohol air dried for a few minutes. If it is greasy it may be advisable to dip it for a few seconds or minutes into benzene.

Large or medium-sized species with moderately heavy wings that have been properly spread require no special precautions in this method. In more delicately winged forms, such as most geometers and some lycaenids, the wings have a tendency to collapse and draw together when they dry, particularly if the bases of the wings were injured in spreading. To correct this, a clean microscope glass slide may be used to pick up the wings from the wash alcohol. While still wet the wings are spread on the slide in the desired position and allowed to dry. If the slide is clean the wings will come off easily when dry.

Micros present a special problem. We have obtained excellent preparations, with the fringes in normal position and all details of venation clearly evident, by supporting the wings with a single piece of cellulose acetate film of 0.25mm thickness (Fig. 2). The film is bent up at a right angle at the base and attached either to the pin or to the cork with a drop of clear finger nail polish (also passed through the pin in simple mounts). It is then bent down at an angle outwardly under the wings to support them. It is important not to extend the support too near the body. The wings may be anchored to the support at the base if desired, but this is usually not necessary if the attachment to the mount is firm. After the specimen is thus prepared the above-outlined procedure for wetting, bleaching, and dehydrating is followed. The supports are left permanently attached to the specimen. Larger species may be handled in the same manner, but they require a thicker celluloid support.

An alternate method for micros, one requiring less care and dexterity, consists of cutting off one set of wings and anchoring them with a small drop of finger nail polish to a celluloid slip. The latter is then handled as above and finally pinned under the remaining parts of the specimen and anchored to the pin with a drop of finger nail polish.



To obtain best results it is advisable to spread the specimens with the hind wing not inserted as far forward under the fore wing as is customary, since it is often necessary to observe details of venation of the anterior portion of the hind wing and posterior portion of the forewing. If conventionally spread specimens are used, proper bleaching of this area is insured if the two wings are lifted apart slightly in these areas while the specimen is in the wetting solution.

This method results in specimens that show details of wing venation nuch more clearly than even stained microscopic preparations. With a little care in bleaching a strongly contrasting venation can be obtained. The left side of the specimen and its body retain the original coloration which may be needed for identification purposes (Fig. 1). In examining the venation in small specimens it is suggested that oblique transmitted light be used for greater contrast. The greatest advantage to the instructor is the ease and speed of preparation as well as the economy in specimens. For quiz purposes entire specimens of common species may be bleached to discourage undue reliance on color patterns. Other details of the anatomy of Lepidoptera obscured by scales may be clarified greatly by bleaching, for example labial palps, patagia, tegulae, tympana, and genitalia.

We believe that this method may have application outside of class material, but it would be hard to convince lepidopterists to use it on valuable collection specimens. The use of bleaching in the preparation of insect material in general has been greatly neglected. We find that in heavily pigmented structures, such as elytra and head capsules of beetles, a much better picture is obtained if bleaching with Clorox is used instead of cleaning with potassium hydroxide. A combination of the two is also very useful for microscopic preparations since it greatly reduces the time needed for clearing the specimen and disturbs less the natural conformation of sclerites. We have not used other bleaching agents, such as hydrogen peroxide. These might prove useful with different types of material.

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