SMITHSONIAN MISCELLANEOUS COLLECTIONS
VOLUME 103, NUMBER 6

ON THE PREPARATION AND PRESERVATION OF INSECTS, WITH PARTICULAR REFERENCE TO COLEOPTERA

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Bureau of Entomology and Plant Quarantine
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It is beyond the scope of the present paper even to enumerate the multiplicity of methods employed and customs adhered to in the preparation of insect specimens for the cabinet. Rather, what is intended is solely a presentation of certain procedures which have proved most useful to the writer after a period of considerable experimentation.

Broadly classified, there are two schools of technique in the mounting of Coleoptera. The European entomologist habitually displays his smaller specimens by gluing each of them, ventral surface down, to a standard rectangular card which is then pinned. The American prefers to mount his at the apices of small cardboard triangles whose bases hold the pins. In the first method, the appendages are protected but only the dorsal aspect of the insect is visible, an examination of its ventral characters necessitating the removal of the specimen from the card. This is a tedious and dangerous routine which the average collector is reluctant to undertake; its avoidance, however, cannot fail to result in identifications based entirely on dorsal anatomy. The American system, on the other hand, while providing opportunity to study lateral and ventral characters (though only those not obscured by the legs in their flexed positions) fails to afford proper protection to the specimen. Neither technique ordinarily includes any degreasing treatment with the result that too often, during the passage of time, escaping oils render the specimen unfit for study.

The following is a description of a third procedure which attempts to combine the merits of both schools and, at the same time, to eliminate the more undesirable features of each. This technique was developed specifically for the purpose of preparing good research material in the Cicindelidae and Carabidae. However, it has proved equally useful in other groups of beetles; and it has been adapted with success to insects of various additional orders, especially to those whose membranous wings, if present, are folded and concealed.

I. KILLING

Since the final results are, in large measure, dependent upon the killing agent used, and the manner in which killing is accomplished, the choice of a suitable lethal chemical and its proper application become matters of the highest importance. In general it may be said that killing by means of the vapors from volatile anesthetics (lipoid solvents) is to be preferred to direct submergence in any fluid. Two such substances are recommended:

a. Carbon tetrachloride.—If specimens do not come into direct contact with the fluid, they will remain relaxed after death, especially if the catch is large and is allowed to remain in the killing bottle at least 24 hours. A full bottle, however, should not be neglected longer, as disintegration of soft parts will soon set in with consequent loss of setae and abdominal segments. A convenient vehicle for carbon tetrachloride is chopped elastic bands; rubber imbibes the fluid readily and retains it a long time. Crumpled paper toweling is also satisfactory, especially when used in connection with light traps requiring the efficient operation of large killing jars at least partly open to the outside air. The heavy fumes arising from a paper towel saturated with carbon tetrachloride will seek the bottom of the jar, which will remain lethal throughout the night.

Advantages:

1. Produces fair to good relaxation. In this respect it is better than either cyanide or alcohol.
2. Assists in the extraction of oils and fats.
3. Prepares the specimen for genitalic examination by causing excessive swelling when subsequently dropped into ether, an event usually resulting in the extrusion of the genital apparatus.

b. Ethyl acetate (acetic ether).—For all general purposes, this is an ideal killing agent. Its advantages when used as such were first pointed out to the author by Prof. Candido Bolivar, whose technique was to half fill a collecting tube with coarsely ground cork moistened (not wet to the point of adhesion) with the ether. Strips of paper or pieces of cotton tape moistened with ethyl acetate and placed in a vial are equally effective and are better for very small specimens. A still more efficient method is to introduce a half inch or so of wet, mixed plaster of paris in the bottom of a tube or vial. Allow the plaster to set; dry it thoroughly in an oven; then saturate it with ethyl acetate, pouring off any excess fluid after complete impregnation. A collecting bottle of this sort may "stand up" under months of use, if not left uncorked. When exhausted, it can be dried again in the oven and recharged with ethyl acetate. It is advisable to allow

the day's catch to remain in the killing bottle at least overnight, in order to insure the maximum relaxing effect of the vapor-filled atmosphere. Insects may thus be preserved, while awaiting mounting, for an indefinite period, especially if they receive an occasional wetting of ethyl acetate. It is better, however, to remove them for drying, degreasing (II), or preserving in fluid (X) before many weeks have passed.

Advantages:

1. Specimens killed by the fumes of ethyl acetate are completely relaxed and retain their flexibility when subsequently degreased in ether (II) or preserved in Barber's fluid (X). Success in mounting such material on a flat, smooth surface, or in arranging appendages after direct pinning, or in relaxing specimens that have dried is far greater than when either cyanide or alcohol has been used as the killing agent.

2. Unlike the average cyanide jar, an ethyl acetate-charged killing bottle acts promptly, permanently, and uniformly over a considerable period of time. Insects show no tendency to revive if they are allowed to remain in the lethal atmosphere a few minutes after all motion has ceased, and delicate Lepidoptera as well as powerfully jawed beetles usually succumb before they can injure themselves or other specimens in the same bottle.

3. No fading or discoloration has so far been observed by the author as a result of killing beetles and their larvae with ethyl acetate. However, the green pigment of certain moths may turn yellow if the specimens are not removed from the killing bottle as soon as dead.

4. The use of ethyl acetate presents no such hazard as does cyanide, which is far more toxic to human beings.

5. Ethyl acetate is an ingredient of the relaxing fluid described below (XI) and a solvent for cellulose cement; hence its use as a killing agent simplifies the field technique by reducing the number of necessary fluids to be carried on a trip of long duration.

II. DEGREASING

The use of some lipoid solvent in the preparation of Coleoptera, especially carabid, cicindelid, and scarabaeid material, is of the greatest importance. Ordinary sulfuric ether [1] (the commercial product) suffices very well. Specimens should be soaked in ether until the

[1] Ether can be conveniently and economically stored in 1-pound cans fitted with small screw caps seated with cork. While in use as a grease solvent, it will keep well in tightly corked homeopathic vials.

fluid ceases to grow yellow owing to dissolved oils, one or two changes of the bath aiding the process when the bulk of material is great. The duration of treatment varies from about 12 hours to a week, depending upon the size and number of specimens per bottle, the volume of ether in proportion to material, and the fat content of the particular insects being degreased. To protect the specimens from the clinging, watery exudate which escapes from them and tends to work its way to the bottom of the container, a small wad of loosely folded absorbent tissue or filter paper should first be placed in the bottle. This will serve to capture such waste.

Ether will preserve ethyl acetate-killed material in a perfectly relaxed condition for an indefinite period. However, a certain degree of surface etching will take place in very oily ether where specimens should never be left very long. Also, very small specimens isolated in a volume of ether too large to be discolored by them may become temporarily brittle if given this treatment for more than a few hours. Should such an event occur, a drop of Barber's fluid (XI) on the dry insect will serve to free its articulations instantly.

Bulky specimens which have swelled in the ether bath should be removed to a pad of absorbent paper where all turgidity can be eliminated by gentle pressure after pricking through the dorsal abdominal and nuchal membranes with a needle. Such material should always be returned to clean ether for further degreasing before mounting.

Very hairy insects [2] and those possessing easily detached scales or farinose areas should be subjected to the ether bath only when the oily nature of the species at hand demands such treatment. Relatively few Coleoptera (certain groups of farinigerous Buprestidae, Curculionidae, etc.) fall under this category. These cannot be ether-treated for long without the loss of at least some of their powdery secretion.

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[2] Degreasing of pilose insects, such as some bees, flies, etc., must be thorough if attempted at all. When body fats have been completely removed, the pile may rather easily be restored to its natural fluffiness by lifting it with a camel's-hair brush while applying a stream of air through a blowpipe. The detachable pile which covers the bodies of moths cannot, of course, withstand rubbing or brushing. Gravid females of large-bodied species, however, often require degreasing because of the high lipoid content of their egg masses; since immersion is to be avoided in such cases, it is therefore desirable, before spreading these, to remove the viscera through an incision in the ventral abdominal wall. By clamping the pin (head end) in a horizontal position, it is possible to perform this operation with the abdomen hanging free. The abdominal cavity should be dusted with cotton dipped in dry plaster, blown clean, and loosely stuffed with fresh cotton.

Advantages:

1. Ether effects the extraction of body fluids as well as complete degreasing. After the ether bath, piceous, castaneous, and fulvous specimens will acquire a remarkable freshness of color, lightly pigmented areas appearing in vivid contrast to dark as soon as evaporation has taken place. Likewise, the true texture of the chitin, a useful habitus character dependent upon clean microsculpture, will be preserved as in life.

2. Never soiled with a sticky layer of grease to which dust and fine litter adhere, the ether-treated specimen can always be cleaned with a dry camel's-hair brush with minimum danger to setae.

3. The greaseless insect can be firmly cemented to the mounting support; should it become dislodged, its lightness and flexibility will insure a good chance of survival intact. Specimens exuding grease after they have been mounted on points or cards invariably discolor the paper and not infrequently work loose; when pinned directly, they usually corrode their pins.

4. Ether-treated specimens acquire atmospheric moisture rapidly and never become brittle under ordinary climatic conditions. In this respect they contrast very favorably with grease-soaked specimens whose ligaments and musculature eventually harden.

5. Swelling is accomplished by means of the ether bath, specimens killed with the fumes of carbon tetrachloride becoming so turgid in ether that usually the aedeagus and frequently its internal sac are extruded (III). This may be a great advantage, insofar at least as small carabids are concerned, since it eliminates the difficult process of dissecting such material. The more moderate distention of ethyl acetate-killed specimens in ether is useful not only in the extraction of genitalia, but also in the preparation of soft-bodied larvae (IX). Soaking in ether, however, will not cause swelling or extrusion of genitalia in previously dried material.

There are, of course, other satisfactory, though perhaps not equally efficient, lipoid solvents which may be used in place of ether. Chloroform, benzol (benzene), and diethyl carbonate all yield good results but tend neither to be imbibed nor to expel body fluids so readily. Xylol (xylene) is pleasant and convenient to use, but its tendency to stiffen articulations and to deposit a whitish film are disadvantages. Chloroform is the only one of these solvents heavy enough to float both the specimens and their extracted body fluids, a fact which makes it desirable to separate the two with a wire screen. Ether, on the other hand, being lightest of all, permits both to sink, while diethyl carbonate, xylol, and benzol effect a more or less temporary

separation, the water alone, at first, falling to the bottom. Of the three last named, diethyl carbonate is the most efficient solvent. It is less volatile than ether and therefore safer to handle.

III. STEPS PREPARATORY TO MOUNTING

When thoroughly degreased and ready for mounting, specimens should be removed from the ether bath to a pad of absorbent tissue, where, if desired, jaws may be separated and genital extrusions completed. Carabid jaws may best be opened by springing them apart by means of fine forceps applied ventrally. Provided the killing technique has been followed as indicated, the jaws will yield readily and the disclosed mouth parts may then be easily cleaned with a soft camel's-hair brush dipped in ether. Partial extrusion of the genital apparatus of males may be successfully completed by slight pressure on the abdomen; should this not produce the desired result, the insect is placed ventral surface uppermost and held while a sharp needle is inserted in a membranous portion of the median lobe and the latter gently extracted. The laterally curved aedeagi of carabids require urging counterclockwise in the direction of their curvature. Any fluids escaping from the body during the process of handling should be washed off in a bath of clean ether. If the specimen is minute, the operation should be performed in fluid (ether or Barber's fluid) under a binocular, a special tool of fine pin wire or drawn glass holding the beetle firmly against the bottom of the dissecting dish (fig. 1).

It is desirable to transfer drying specimens to a smooth, clean surface (preferably glass) for the final arrangement of their appendages. In preparation for the slip method of mounting, the legs are oriented close to and on a plane with the body, and the antennae are directed backward along the sides. Until cleared mounts of genitalia are desired, these organs had best be left attached to the abdomen; in male Carabidae and Cicindelidae, they tend to orient pointing clockwise as seen from above and in this position they are most convenient for study.

IV. MOUNTING ON TRANSPARENT SLIPS

A sufficient series of each species collected at one time and under the same ecological conditions are assembled and are ready for mounting as soon as their surfaces are dry. Mounting should be done before the insects have become stiff, since the legs in drying tend to elevate the body slightly and this necessitates relaxing the specimens again before they can be properly cemented to a plane

surface. Should this eventuality arise, the dried insects can be completely and instantaneously relaxed merely by dipping them in Barber's fluid (XI).

The foregoing technique is preliminary to mounting on a transparent supporting surface, though it does not preclude pinning in the usual manner. The former, or slip system, applicable to large specimens as well as small, has been developed in two ways:

a. Cellulose acetate mounting.—Only the best quality, heavy (.015, .020, .025 inch = 15, 20, 25 gauge) acetate sheeting [3] can be used to

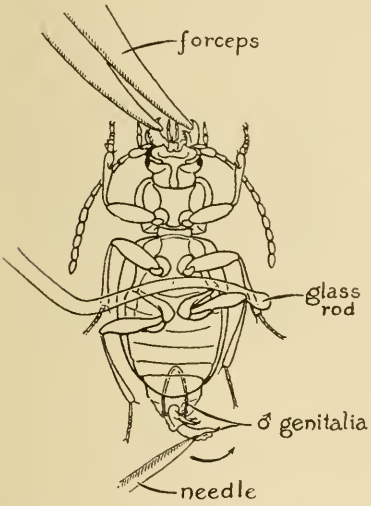

Fig. 1

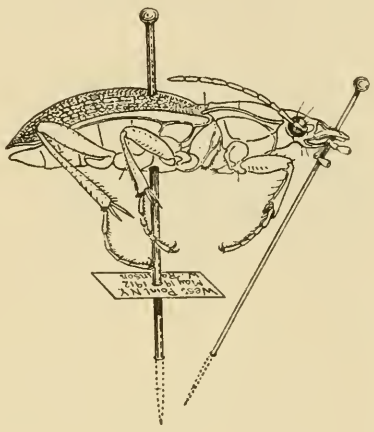

Fig. 2

FIG. 1.—Preparing a minute male carabid for mounting.
FIG. 2.—Preferred arrangement of parts in a pinned carabid.

advantage. This material, unlike celluloid, will not curl when pinned nor, apparently, will it discolor or lose any of its transparency when exposed to the light. Up to 25 gauge, it can be cut easily with ordinary scissors, can be punctured with a No. 3 pin, and will grip the latter firmly without need of reinforcement.

On a piece of acetate sheeting of convenient size and gauge, each species-time-locality series is cemented separately in a compact group, usually of one row. Adequate space is left between the groups so

[3] Acetate sheeting ordinarily tends to fog by collecting droplets of an oily fluid when in contact with the fumes of pest and mold repellents such as naphthalene, paradichlorbenzene, phenol, creosote, etc. Carbon tetrachloride, however, has no such effect on it. Sheeting which will *not* fog under the above conditions can be obtained from Eastman Kodak Company, Rochester, N. Y.

that they may be cut apart without danger to appendages; and an ample margin of free sheeting should remain in back of each group in order that the pin, which is placed there, may not interfere with hand-lens observations of the specimens nearest it (fig. 3). Standard strips of 15-gauge sheeting cut in $\frac{1}{2}$- and $\frac{3}{8}$-inch (13-mm. and 10-mm.) widths will greatly facilitate the mounting of small to minute specimens and will lend a neat appearance to the collection. The beetles should face and be close to a long edge; the slips bearing series or single specimens can then be speedily cut apart in one operation yielding mounts of uniform dimension from back to front with the specimens thereon occupying similar relative positions. If sufficiently heavy sheeting is used for large beetles, it is seldom necessary to

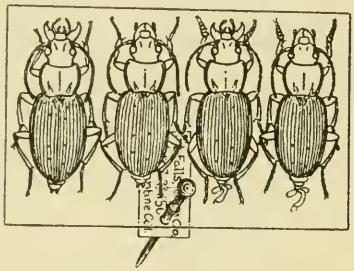

Fig. 3

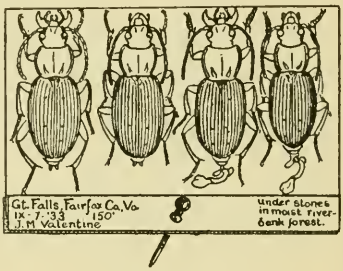

Fig. 4

FIG. 3.—The cellulose acetate slip method of mounting in series.
FIG. 4.—The cover glass slip method of mounting in series.

place the pin in any position other than the standard one, in the center of the rear of the mount. Should, however, an extra large mount require reinforcement, a drop of cement placed at the point of exit of the pin will suffice.

A good-quality, clear cement of cellulose base [4] should be used. It should not be applied straight from the container, however, but should first be diluted with sufficient solvent to allow small drops to form slowly on the head of a pin after the mixture has been thoroughly stirred. At least two lots should be made up, one diluted with ethyl acetate and the other with amyl acetate. The former is relatively quick-drying and is used in cementing firmly all but the smallest specimens to their acetate mounts; the latter is slower to dry and therefore more useful in attaching minute specimens and in mending broken appendages. A convenient applicator is the head of a long, fine insect pin stuck into the cork of the vial in which

[4] Duco "household" cement has been found very serviceable.

the cement is mixed. Good adhesion with a minimum droplet of cement depends, of course, upon the consistency of the latter, which should not be so thick as to provide poor capillarity, nor so thin as to spread beyond the original confines of the droplet.

Advantages:

1. An assemblage of specimens illustrative of a circumscribed population or of an ecological sample of a species provides far more valuable data than isolated specimens and should be kept intact under the same label. The slip method of mounting makes this possible for large specimens as well as small, and promotes comparison of individuals.

2. Too often the customary procedure of mounting small, flexed insects on cardboard points results in specimens whose appendages and ventral anatomy are seriously obscured by the adhesive. Another handicap to study frequently arises from undue arching of the body and depressing of the head. The transparent-slip technique allows maximum visibility of all parts, which, in all but the most convex forms (some weevils, mordellids, etc.), can easily be arranged so as to appear nearly on one plane. Under these conditions, buccal and appendicular anatomy and comparative dimensions of legs, etc., can be observed with a maximum degree of ease.

3. One of the commonest causes of damage in a collection is the inadvertent contact between protruding labels and specimens delicately mounted on points. The slip method obviates this hazard.

4. Mutilation of specimens and interference with hand-lens examination by pins thrust through elytra are eliminated.

5. Labels under acetate mounts are readable from above; usually they can be larger than the minimum-sized label customarily attached to specimens mounted individually.

6. A great saving of time is effected when specimens are mounted in series by reducing the number of labels necessary and speeding up transfer.

7. Space is conserved through the close approximation of specimens on the mounting slip.

8. Far fewer pins are used.

9. The problem of which method of mounting—pin or point—should be applied to a rather small specimen is eliminated; this makes for uniformity in the collection.

10. Any specimen can instantly be removed from the acetate mount merely by touching it with a brush containing ethyl acetate.

b. Cover-glass mounting.—This is a refinement of the above technique, micro cover glass (No. 1 or No. 2) being used in place of

acetate sheeting. Cover glass is best cut with a diamond point; a small chip cemented to the end of a drawn-glass tube makes a perfect instrument for the purpose. The slip may be cut to the required size after the specimen or series has been attached to it. It is imperative to work on a perfectly smooth, level surface, preferably plate glass; and to use a rule which will not slip, such as a microscope slide faced on one side with adhesive tape. The best adhesive for mounting on glass is acetate cement thinned with ethyl acetate. A generous application of a slightly thicker mixture should be used to attach two narrow strips of bond paper or single-ply bristol board (about $\frac{1}{8}$ inch wide for small slips), one on each side of the glass along the entire rear margin of the mount. Approximately half the width of these strips should be occupied by the glass between them, the other half being left for direct contact of the strips and subsequent perforation by the mounting pin. The strips should be gently pressed together and carefully aligned. When working with a quantity of material of fairly uniform size, a number of slips can be attached at intervals to a long bottom strip, and a top strip of equal width can then be cemented over the whole. After 15 minutes or so of drying, before the cement has become thoroughly hardened, the mounts should be cut apart, the paper trimmed close to the glass with fine scissors, and each mount carefully pinned on a flat-topped, gauged block, preferably slotted (VI) (fig. 4). It is advisable to pin the mount tilted slightly upward, and to immobilize it with a drop of cement deposited on the bottom strip around the pin.

A well-constructed cover-glass mount is neat, strong, and optically ideal. However, unless its specimens have been exceedingly thoroughly degreased, it may, in time, collect fine droplets of oil emanating from them. Cover glass is, therefore, best employed in mounting small specimens (10 mm. or less) which can easily be cleaned of all grease.

The writer has experimented with a variety of materials for the supporting strips of these mounts. None of the transparent "plastics" and celluloids used has proved so efficient as heavy paper. Besides its stability, another advantage in the paper base is that it can effectively serve to display data, numbers, etc., written thereon.

V. DIRECT PINNING

Provided specimens are of sufficient size to justify impaling them on insect pins, they can always be effectively mounted in accordance with this standard technique. It should be pointed out, however, that substituting direct pinning in the larger forms for the transparent-

slip method entails some sacrifice of utility, safety, and uniformity. On the other hand, an obvious advantage in pinning lies in the resulting mobility of individual specimens. Whichever method is chosen, the quality of the finished product depends, to a large extent, upon two factors: proper killing (I) and adequate degreasing (II).

In mounting Coleoptera, the location of the pin is internationally agreed upon: It should enter the anterior discal portion of the right elytron and emerge on the right side between the mid and hind legs, passing through the metasternum laterad of the mid line; it should be so oriented that both longitudinal and transverse axes of the beetle are at right angles to it. The writer recommends the European system of appendage arrangement as combining neatness and compactness with maximum visibility. The head and pronotum are extended, with antennae close to the dorsal surface and directed straight back; the legs are directed downward and toward the pin, but with femora more or less horizontal and close to the body, the anterior femora pointing forward, the mid and hind pairs pointing backward. A fresh specimen naturally assumes this attitude when pinched laterally between the fingers. If the insect has been killed with ethyl acetate, its appendages will usually dry in position without the necessity of guard pins. However, drooping of the head and pronotum may occur. This can be remedied easily by resting the jaws (open if possible) of the drying specimen on an outwardly inclined pin placed before it (fig. 2).

VI. GAUGING

Mounting specimens at an approximately uniform height not only makes for neatness in the collection but facilitates microscopic comparisons. Two or three standard heights should be decided upon as proper for corresponding categories of beetles of different body depths, and a gauge constructed which will automatically elevate the mounting support to the desired distance on the pin.

A glass-topped, slotted pinning block greatly facilitates both pinning and gauging. It can easily be constructed of plaster in a mold slightly larger (about $\frac{1}{2}$ inch on each side) than the future glass working surface. The latter is composed of two pieces of plate glass, each 3 inches square, aligned along one edge but held slightly apart by two narrow strips cut from the ends of a microscope slide, inserted in the slot between the plates at the corners, and cemented there in an upright position. The thickness of these lateral pieces should gauge the width of the slot just to accommodate the shaft of a No. 5 insect pin. Two microscope slides are then placed at right

angles with the top plates, flush with and on each side of the slot and contiguous with the lateral pieces to which they are cemented. The space between the slides from the ends of the lateral pieces to the longitudinal free margins of the slides (a distance equal to the thickness of the top plates) is filled by a strip of appropriate width cut the full length of a slide and cemented in place to form the floor of the slot. If preferred, a strip of hard wood, to cushion the points of the pins, may be substituted here for glass. The slot depth, from the free surface of the top plates to the inner edge of the bottom strip, now measures the width of a microscope slide (about 1 inch)

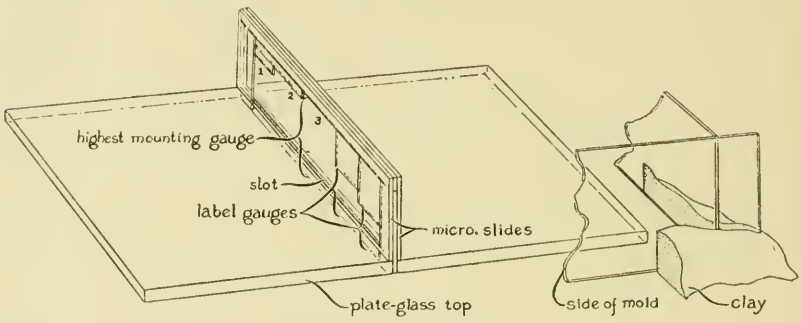

Fig. 5

A slotted pinning block in process of construction.

and gauges the correct position for slips bearing medium to small beetles on the standard 34-mm. ($1\frac{3}{8}$-inch) pin. Additional gauges for lower levels of the mounting slip, as well as for pin-label heights, may be had simply by inserting small removable glass rectangles of the proper widths at the ends of the slots, an operation which had best be performed after the block is otherwise completed. The glass assemblage is now placed, top downward, in the center of the mold and mixed plaster of paris poured over it to a depth greater than the walls of the slot (fig. 5).

A slotted pinning block makes it possible to pin mounting slips squarely with reference to the transverse axis, yet with the longitudinal axis at a slight angle to the horizontal. It is advisable, when pinning heavy mounts, to tilt them slightly upward in front in order to compensate for gravity, and to strengthen them by means of a drop of cement placed so as to embrace the pin at its point of exit.

VII. LABELING

There exists an established precedent of long standing that pin labels, when oriented parallel to the specimen, should face to the right. This custom originated at a time when hand-lens observations were the rule; its function was to insure legibility of labels when specimens were examined in the left hand, the right remaining free for manipulation of the lens. Since the binocular dissecting microscope has come into almost universal usage in entomology and the lifting of specimens with the right hand is, ordinarily, the safer procedure, it follows that a system which would permit labels on specimens held in the right hand to be read without rotating them is to be preferred. Consequently, the writer recommends pinning labels for slip mounts through the right end so that the printing will face left. Additional reasons for this procedure are these: There is usually a pinning space in the uneven right-hand margin of a label; and also, any identification label thus oriented may, when desired, be swung into the standard, left-hand position without becoming inverted. Labels on specimens pinned individually, without mounting support, had best be pierced through the center and oriented facing left, parallel to the specimen.

Double-ply Reynolds bristol board or single-ply Strathmore makes an admirable label paper of the proper thickness to grip the pin effectively. Should a label work loose, a drop of acetate cement on the under surface around the pin will serve to fasten it securely.

VIII. MOUNTING DISSECTIONS

Preparations of mouth parts, legs, genitalia, etc., may be attached dry to the mount bearing the specimens which yielded them. Should a transparent preparation be required, the subject, partly dried on absorbent paper, can be both cleared and dehydrated in xylol, and from this transferred directly to a drop of balsam on the glass or acetate mounting slip close to the specimen to which it belongs. After proper orientation of the dissection by means of a needle, a tiny square of cover glass, or thin acetate sheeting (7.5 gauge), is placed over it. Another method, useful in attaching cleared preparations to specimens mounted individually, is to prepare a small balsam mount on one end of a rectangle of acetate sheeting and run the pin through the opposite end. When not being examined, such a mount may be swung out of the way under the specimen.

Bulkier genitalia, which require examination in more than one plane, may be conveniently prepared for study as cleared objects

by placing them (after dehydration in strong alcohol and clearing in xylol) in small sections of glass tubing drawn to an appropriate gauge and filled with balsam or, better still, with a color-stable gum damar. One end of the tube is left open for addition of more mounting medium as contraction takes place; the other end is closed with a tiny cork plug, or by embedding it in a small cork block in which a suitable recess has been cut with a cork borer. Through the free end of the cork is thrust the pin of the specimen which yielded the dissection. The tube should eventually be sealed with thick acetate cement. Examination of its contents should be made in xylol or cedar oil, where virtually all optical interference due to the curved glass will be eliminated.

The presence of small air pockets trapped within or adjacent to a dissection in a freshly made balsam mount need not be viewed with concern; after a few hours, these will disappear.

IX. MOUNTING LARVAE

Soft-bodied forms, especially the larvae of Coleoptera, have been successfully treated exactly as the adults and mounted with them on the same slips. There is but one departure in technique: While drying, after having been thoroughly soaked in ether, the specimen is warmed under an electric lamp sufficiently to cause expansion of the gas within its body and consequent distention. The heat must be carefully applied and maintained for a few minutes until the specimen has dried in an inflated condition. Too sudden an increase in heat will cause rupture, too gentle an application will cause collapse of the body.[5]

X. CARE OF ACCESSORY MATERIAL

a. Dry storage.—The flat tin boxes in which 50 cigarettes are sold provide admirable storage facilities for Coleoptera. They may be made to do double duty if both top and bottom are utilized. After the metal is first scored with a sharp instrument, two pieces of heavy, smooth cardboard are attached, one to each of the inner surfaces, by means of a suitable cement (such as "Metallic X"). To these cards the ethyl acetate-killed, ether-treated specimens are cemented in close array, with legs at the sides and antennae pointing backward. This should be done while the beetles are still relaxed, and the ad-

[5] This method has yielded excellent results in the preparation of degreased and dehydrated lepidopterous larvae of both smooth and hairy species.

hesive employed should be acetate cement, somewhat diluted with ethyl acetate. Each lot, representing a day's catch or an ecological aggregate, is circumscribed with an ink line and labeled with the date and locality, or with the date plus some symbol referring to a category in the chronologically arranged notes. The boxes are stored chronologically in cardboard filing cases (12 by 6½ by 5 inches), where they stand vertically, hinge uppermost, each bearing an adhesive-tape label along its upper edge. If the cementing has been carefully done and the specimens are clean, the chances of their breaking loose, even in shipping, are extremely remote. Space for naphthalene on the bottom of the filing case is provided by the recess in which the filing mechanism operates. However, should dermestids succeed in entering the tin boxes, they can be destroyed without disturbing the contents simply by dousing specimens and all with carbon tetrachloride and shutting the lids for a few hours.

The chief advantages gained by the above technique lie in the visibility of the specimens and in the compactness of their arrangement. If at any time a beetle is required for mounting, it may be detached immediately from the cardboard after an application, by brush or pipette, of ethyl acetate. It should then be immersed in the relaxing bath (XI) where any residual cement will be dissolved.

b. Preservation in fluid.—Adequate preservation of soft parts with minimum hardening of tissues and stiffening of joints are the criteria for a good entomological preservative. In the writer's opinion, Barber's relaxing mixture (XI) meets these requirements better than any preservative in common use. Ethyl acetate-killed material, stored in this fluid either before or after ether treatment, has emerged years later in an excellent state of preservation and perfectly relaxed. It has proved particularly useful as a preservative or softener for carabid beetles captured in molasses traps, the specimens being soaked in the fluid after having been thoroughly washed and partly dried. In using Barber's fluid as a preservative over a long period of time, it is important to remember to change the supply as often as it becomes darkened by dissolved oils. When the bulk of material is great, several changes may be required.

Because of their hardening properties, neither alcohol nor formalin should be employed alone as a preservative when flexibility of articulations is prerequisite to the mounting technique adopted. Furthermore, the use of these fluids in killing and preserving seems to inhibit, somewhat, the action of degreasing agents. However, the ease with which specimens can be collected and preserved in alcohol frequently justifies its use, especially when time, simplicity of method,

and quantity of material are considerations, or when a hard fixation of perishable internal organ systems is desired for purposes of dissection. If alcohol is used for preserving Coleoptera, strengths neither exceeding 70 percent nor less than 50 percent are recommended.

XI. RELAXING

The writer has found the relaxing mixture developed at the United States National Museum by Herbert S. Barber to be extremely efficient and versatile. In this fluid, ethyl acetate-killed specimens become plastic almost instantly and genitalic dissections may be made a very short time after immersion. It is invaluable for rejuvenating old, greasy specimens, and will dissolve every mounting adhesive now in common use. The formula is quoted below:

Barber's fluid

Ethyl alcohol (95 percent)	265 parts
Water	245 parts
Ethyl acetate (acetic ether)	95 parts
Benzol (benzene)	35 parts

Should the benzol separate out, a little alcohol, added slowly with shaking, will serve to bring it back into the mixture.

Relaxing alcohol-killed material preparatory to slip mounting presents a difficult problem. However, if such specimens are completely dried under a lamp, then slightly moistened with Barber's fluid and quickly blotted, recalcitrant appendages can usually be made to remain in the desired positions. A simpler and perhaps more efficient method consists in a thorough soaking (several hours at least) in chloroform. Articulations then become fairly easy to manage, especially in the smaller specimens whose appendages, while they are still moist, readily yield to manipulation with needles.

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