

more than 33 per cent. and that ten years thence there would not be a full-blooded Marquesan alive. Once populous valleys are already swallowed up by the tropical jungle.

It is a well-known fact that cultivated plants can not successfully compete with the wild vegetation when the protecting arm of man is removed. And as the Marquesan is doomed to extinction, so will his breadfruits—by travelers described as superior to all others of their kind—inevitably follow if man does not intervene. Some of these varieties may have become extinct already or be near extinction. In the Society Islands the situation is but slightly better. I quote as follows from a letter recently received from a correspondent in the Fiji Islands: "The Tongan colonists in these islands seem to be the only people that are giving any attention at all to the breadfruit. I fear that this splendid fruit is gradually being permitted to die out."

While the disappearance of the breadfruits would be an economic loss, there would be, in addition, the sentiment of the loss of that which has been the staff of life of one of the races of man which our own civilization had destroyed. Nor should it be forgotten that while in the sciences and trades a lost art or a lost invention may be rediscovered, in the plant world this is not so, for when the last individual of a species or a variety has passed away, it is irrevocably lost. Again, for all that has been written about the breadfruit and the multiplicity of its forms, the curious fact remains that not more than three varieties appear to have found their way from the South Sea archipelagoes to other lands. Finally, as has already been stated, the gathering together of the breadfruit varieties in the Pacific archipelagoes for a comparative study should add further evidence relative to the much mooted question of the migrations of the peoples within those regions.

Various writers and correspondents have quoted more than 100 variety names of the breadfruit. With a liberal allowance for synonyms there must be at least 35 varieties.

I estimate that an expedition to the Marquesas and Tahiti covering a year would be sufficient to get together the largest number of varieties (including the most valuable ones) at the least expenditure, and that this would cost \$8,500. I estimate that it would require three years' work to gather together plants of all the breadfruits in the Pacific, including the Society Islands, Marquesas, Samoa, the Fiji Islands and the Caroline Islands. The expense is estimated at \$24,000.

Correspondence relative to the subject is invited.

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LABORATORY APPARATUS AND METHODS

DRIED PREPARATIONS OF EARTHWORMS¹

For many years the usual method of studying the structure of the earthworm has been to dissect it wet and to study thin sections of it. This method will doubtless remain the standard. However, I have recently discovered that earthworms, if properly freed from ingested soil, fixed, dehydrated in alcohol and then dried can be readily cut in such a manner as to reveal in a strikingly clear fashion all the structures usually studied, except the histological details. Indeed, these preparations show many details not usually seen in wet dissections or in sections. In brief, the method of preparing the worms is as follows:

Free the worms from their ingested materials without permitting them to eat filter paper or paper towel, since these fibers are difficult to cut when dried. Anesthetize in any approved manner; fix and harden by immersion in, or by injection with, a chromic acid solution (2 to 5 per cent.); lay them out straight in the fixing solution and allow them to harden for 12 to 24 hours; wash for an equal time or longer in running water; dehydrate with alcohol; then permit them to dry at room temperature. Prolonged preservation in alcohol tends to bleach them and the addition of terpineol (1 part to 19 of 95 per cent. alcohol) has a marked bleaching effect. This is desirable for some types of preparation. In drying, some of the worms shrink somewhat, but many of them, if well hardened, do not shrivel, but the muscular layers become thinner. Dried worms, thus prepared, have a tough, leathery consistency which is firm but not brittle. They can be relaxed in a moist chamber just as insects are relaxed either before or after the dissections are made.

The external features that may be seen as well or better in the dried worm than in the wet are the setae, somewhat abnormally protruding owing to the thinning of the body wall, the nephridial pores, dorsal pores, openings of the receptacula seminis and oviducts. Openings of the vasa deferentia do not show so well as in the wet worms.

To dissect the dried worm hold it in the fingers and cut away portions of the body wall, or split the worm or pieces of it on or near the mid-dorso-ventral plane, using a sharp, thin-bladed scalpel or safety razor blade. The cutting must be done with a clean stroke with a slanting cutting edge. The blade will require frequent honing or stropping. Shaving away successive thin slices usually results in shattering the tissues. A useful preparation, but difficult to make, may be made by slicing away the body wall of the

¹ Contribution from the Zoological Laboratory of the University of Michigan.

sides and dorsum of the anterior 15 to 20 somites. Then the septa or any other parts which obscure the desired structures should be picked away by means of fine-pointed forceps, the points of which have been made sharper by filing or grinding. This work with forceps must always be done under a binocular microscope, and brilliant illumination must be employed. The hearts and certain other parts, if broken in the process of dissection, may be cemented in place by means of white shellac, or euparal.

One of the easiest preparations to make and, at the same time, most useful is made by splitting the anterior 17 to 20 somites in the mid-dorso-ventral plane. Such a preparation reveals all the parts of the digestive system from mouth to intestine. The muscles radiating out from the dorsal wall of the pharynx to the body wall are shown here better than in any wet dissection, or in sections. The openings of the calciferous glands are revealed as large openings a short distance anterior to the crop. Other organs which may be readily identified by any student are the brain, ventral nerve cord, ventral and dorsal blood vessels, parts of nephridia, seminal receptacles and septa. Posterior to the gizzard the mesentery supporting the sub-intestinal bloodvessel may frequently be seen as a plate of thin tissue traversed by branches of the sub-intestinal vessel. In preparations similar to the above the esophagus may be dissected out by means of the fine-pointed forceps. This will lay bare complete nephridia, seminal vesicles, seminal receptacles and many of the finer ramifications of blood vessels. From such preparations the student can gain a better appreciation of the coelom than can otherwise be had. Other useful preparations may be made by cutting parallel to the ventral surface, either above or below the nerve cord which may then be seen with its ganglionic swellings and frequently with unbroken nerves. If the cut is below the nerve cord the subneural blood vessel may be seen applied to the cord with its branches extending along the ventral surface of the nerves.

To show the relationship of setae to the body wall, the muscles which tilt the setae, and the muscle which unites the tip of the ventro-lateral pair of setae with the lateral pair of each half somite cut a strip of the lateral body wall so as to include the two rows of setae and view it from the inner surface. No other kind of preparation that I know of shows these structures so well. Many other dissections have been made, but those described above are sufficient to illustrate the method and to suggest possibilities to the experienced laboratory worker.

The dried preparations have several advantages over wet dissections. Dry surfaces do not reflect the light as do wet surfaces. Outlines appear sharp, there being no fuzziness of the image due to reflections below, or from, the surface of the water. By

using a variety of dry preparations, structures can be seen from different points of view than in conventional dissections. This is especially valuable after the student has made his dissection and has studied cross sections. The making of the dried preparations is not difficult, nor especially time consuming, and their preservation is simple. They may be studied with the simple dissecting microscope or with the binocular.

The dissections of dried forms may be mounted on blackened blocks of wood, using glue or shellac as the adhesive, or they may be glued to black paper which has been glued to small blocks of wood. Under no consideration should the preparations be mounted under glass, since reflections from the glass, dust on its surfaces and absorption of light by the glass interfere seriously with good vision. It is better to make new preparations from time to time than to protect them with glass coverings.

Further refinements of this method remain to be worked out and it is hoped to report on them at a later time.

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A SIMPLE SHAKING APPARATUS FOR USE IN ENZYME STUDIES¹

In the course of our digestion experiments with castor bean lipase, it was found necessary to devise a shaking apparatus in order to overcome certain conditions that prevented concordant results from being obtained from day to day.

The apparatus used by us, as shown in Figure 1, has given very satisfactory results in our enzyme studies and was constructed as follows:

An old incubator was heated by means of a carbon lamp, the temperature being controlled by means of an electric thermoregulator with condenser. A thermograph was attached to record temperature fluctuations that were found never to exceed $\pm .5^{\circ}$ C. A grooved wooden wheel ten inches wide and one half inch thick was employed to hold the bottles containing the digestion mix. These bottles were of five cc capacity and were prepared from hard glass ignition tubes of 13 cc capacity. They were held in place by means of copper wire extending through minute perforations in the wheel, the base of the bottles resting in small borings extending partly through the wheel, the dimension of the borings approximating that of the bottles themselves. The wheel was allowed to revolve at 60-70 R. P. M., the power being supplied by a small motor placed outside the incubator and controlled by means of a rheostat. In order to eliminate

¹ Contribution No. 3 from the Department of Agricultural Chemistry, the Pennsylvania State College. The drawing is by Mr. Walter Trainer.