The use of a proteolytic enzyme in clearing genital preparations

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Abstract: A method is described for clearing insect genitalia preparations from adhering soft tissues by means of a bacterial proteolytic enzyme. This technique is much less dangerous for eyes, clothes and optical instruments than boiling in potassium hydroxide.

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Introduction

Already about two decades I am using a method for eliminating soft tissues remains from chitineous genitalial structures that is very successful and has many advantages over boiling in potassium hydroxide. The method has been used by a small number of colleagues, but was never published because of the lack of general availability of the main chemical. All those that have used the method are very enthousiastic about it, and some wanted to mention it in forthcoming publications. Thanks to the licensee, the enzyme in question is now available for biological applications, and the method therefore can be published now for a more general use.

The proteolytic enzyme used is of bacterial origin, and is widely used in laundry detergents. The enzyme has a good effectivity even at room temperature, contrary to similar enzymes that originate from warmblooded animals. The optimum pH is between 9.5 and 10. The temperature optimum is about 60 °C; at temperatures above 65 °C the enzyme is gradually inactivated. The enzyme has been granulated to prevent inhalation of dust, and is highly concentrated. The cost of the enzym is low, less than US$ 0.01 per preparation. The formulation that is described here is distributed under the name "Genitase" in two gram vials by the firm "Biopraxis", Irenestraat 8, 2351 GL Leiderdorp, The Netherlands.

Usage

In a small cup that can be covered, like an excavated glassblock, about 7 drops of tapwater and a drop of household detergent are pipetted. A few crystals of potassium carbonate (about the tip of a lancetshaped preparation needle) are added to create an alkaline environment. Too much potassium carbonate will cause flocculation of the detergent (this can be restored by adding a few more drops of water). Then 4 to 7 grains of enzyme are added, depending of their size. Within about a minute the grains dissolve into a turbid solution.

The solution is stirred, the genitalia to be cleared are added, and the cup is covered to prevent desiccation. The cup is kept overnight at room temperature; next morning adhering muscles and connective tissue will have been digested. Membranes that possess chitineous structures are left intact. The clearing process is accelerated at higher ambient temperatures. The genitals may be kept in the enzyme suspension without harm for several days. The genitalia finally are transferred to a drop of water, where possible tissue remains may be removed under the binocular microscope with a pair of fine hooks (made of steel points pinched in needleholders).

For the processing of large series of genitalia the small plastic trays that are used in bloodgroup assessment are very useful. They contain eight numbered cavities.

Discussion

The method described here has proved to be very effective in over a thousand genital preparations of histerid beetles that had been killed with ethyl acetate. The method was successful as well for old museum material. In some old museum specimens, however, the soft tissues had been transformed into a more or less homogeneous orangebrown waxy mass, possibly by the killing agent used or the subsequent fixation. In these cases the method is much less successful.

The enzyme method has many advantages over boiling in potassium hydroxide solution. It is much less dangerous, both for the eyes and cloths and for the objects that are treated, the objects may be handled if needed with fine brushes (as the enzyme does not dissolve the hairs, like KOH does), and the clearing process may be safely followed under a binocular microscope.

It may be expected that this method will work well with all old or fresh insect specimens that have been killed with ethyl acetate, and probably in most cases where boiling in a potassium hydroxide solution has been customary, e.g. radula preparations of molluscs.

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