method properly. More precise definitions of varieties of biological information appear necessary.

Zusammenfassung. THEODORIDIS und STARK[1] haben vorgeschlagen, dass der Informationsinhalt der Biosphäre ein objektives Kriterium des Evolutionsfortschritts schafft. In dieser Aufzeichnung prüfe ich diesen Informationsbegriff nach und schliesse daraus: 1. dass ein zuständiges Mass noch nicht vorhanden ist; 2. dass die Informationsbeweise in der genetischen Evolution irreführend sein mögen; und 3. dass wir vorerst die folgenden Parameter zu bestimmen und zu messen versuchen sollten: die Gesamtinformationen der Biosphäre; die mindeste Obergrenze der Eingangsinformationen, die für jede besondere Lage der Biosphäre unentbehrlich ist; und die überholte Informationenmenge in dem Genom der einzelnen verschiedenen Spezies.

O. MAYO

Biometry Section, Waite Agricultural Research Institute, University of Adelaide, 5064, Adelaide (South Australia), 24 September 1971.

PRO EXPERIMENTIS

Time Saving Improvements in the *Galleria* Bioassay for Juvenile Hormone

The great wax moth (*Galleria mellonella*) bioassay for juvenile hormone[1] is the most sensitive so far known, but it has the disadvantage that the whole procedure, from collecting the pupae until the application of the hormone sample, lasts rather long. As a consequence only a limited number of samples or extracts can be bioassayed per day.

Breeding wax moths in large quantities is easy and not time consuming[2]. DE WILDE et al.[3] described a method for obtaining pupae with thin cocoons, using pieces of plastic tube, 3 cm long and with an inner diameter of 4 mm. This procedure has been simplified by Dr. Schooneveld, Wageningen. The Netherlands (personal communication), using siliconized glass tubes of the same diameter which allow a quick removing of the cocoons from the tubes. The bottle neck of the bioassay is setting free the pupae from their cocoon. Although the cocoon is thin when the larvae are forced to spin in the 4 mm glass tubes, it takes a long time to open the cocoons by forceps and always a rather high percentage of the pupae are damaged and useless in the bioassay. A second difficult step in the procedure is the application of the hormone sample on the pupae. Hitherto, this was done by scraping a small amount of the hormone-paraffin-olive oil mixture with a blunt mounted needle and melting it over a flame and applying it to the wound just before the mixture hardens again by cooling. Only very well trained assistants can carry out this step without causing burns to the pupae.

We have developed simple techniques to overcome these two difficulties. First, a trick is described to remove the pupae from their cocoons very quickly without damaging them. Secondly a device was designed for the application of the hormone-paraffin-olive oil sample which completely avoids burns.

Materials and method. Setting free the pupae from their cocoon. Prepare a solution of 40 gm NaOH in water (1*N*). A sieve in plastic (small sieve for milk, available in ordinary shops, is very well suited). Beaker for the NaOH solution and 2 petri dishes or any other shallow containers, filled with water. Filter paper.

Procedure (Figure 1). Put groups of 5 to 10 pupae with cocoons in the sieve. Bring the sieve in the NaOH solution and immerse the cocoons. They begin to dissolve within a few seconds. After about 15–30 sec, the thinnest parts of the cocoons are dissolved. The sieve with the pupae is removed from the NaOH solution and it is turned over so that the pupae fall into the first petri dish with water. With a glass rod the pupae are turned around in the water to remove most of the NaOH and subsequently the undissolved parts of the cocoons are removed by hand. After this step the pupae are picked up again with the sieve and thrown in the second petri dish filled with water and they stay there for 2–3 min to remove the rest of the NaOH. Then they are put on filter paper to dry. Although this procedure may seem very drastic, it is not at all harmful to the pupae. Even pupae which remain for 5 min in the NaOH solution are not damaged. About 5% of the pupae are mechanically damaged during this procedure, but this is less than when the cocoons are removed by forceps. This

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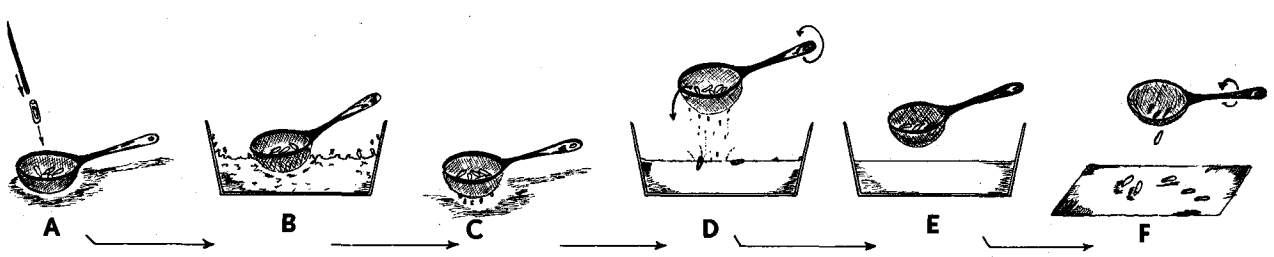

Fig. 1. A) Remove the cocoons from the siliconised glass tubes and put them in a plastic sieve. B) immerse the cocoons in the NaOH solution. C) Shake the sieve to remove most of the NaOH. D) Throw the pupae in H_2O and remove the undissolved parts of cocoons by hand. E) Pick the pupae up with the sieve, wash them in H_2O for 2–3 min. F) Dry the pupae on filter paper.

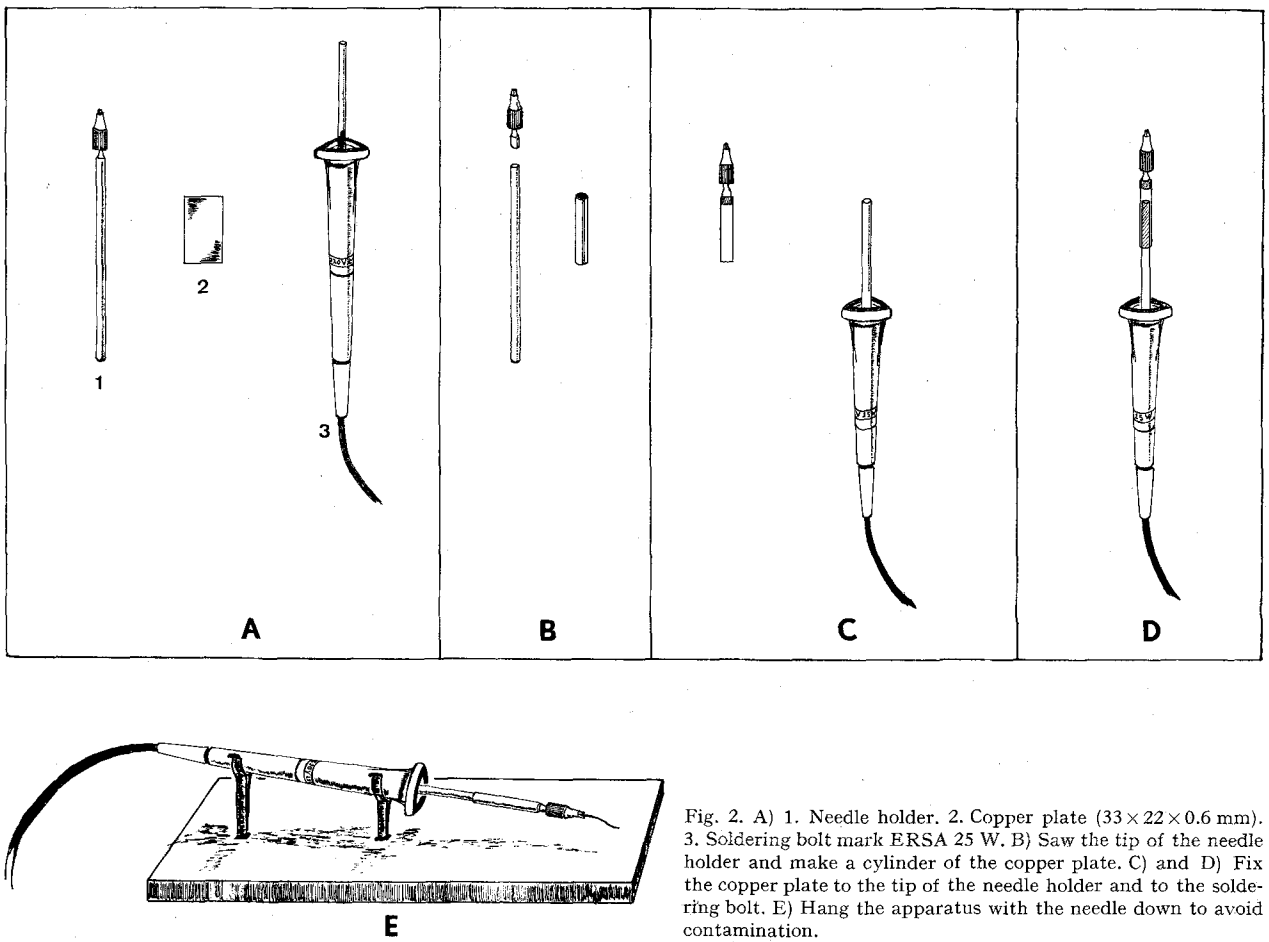

Fig. 2. A) 1. Needle holder. 2. Copper plate (33 × 22 × 0.6 mm). 3. Soldering bolt mark ERSA 25 W. B) Saw the tip of the needle holder and make a cylinder of the copper plate. C) and D) Fix the copper plate to the tip of the needle holder and to the soldering bolt. E) Hang the apparatus with the needle down to avoid contamination.

procedure is about 4–5 times less time-consuming than the forceps method. 200–300 pupae can be set free from their cocoon in 1 h.

A device for the application of the hormone sample. Soldering bolt, mark ERSA multityp 25 W, available in shops for spare parts for radio and television etc. Price in Belgium about 6 dollars.

Inoculation needle-holder in metal (copper, stainless steel). The diameter of the handle should be about 5.5 mm. A small plate in copper (about 33 mm × 22 mm × 0.6 mm). It will be used to fix the needle holder to the soldering bolt. Copper wire, 0.8 mm thick (will be used as needles). An adjustable electric resistance.

Procedure. (Figure 2). Saw the handle of the needle holder so that the top part is 3 cm long. Link this part to the tip of the soldering bolt by means of the copper plate. Connect the soldering bolt to the adjustable resistance and then to the current. Cover the metal parts of the soldering bolt and the copper plate with a tube in bakelite or any other material which isolates heat. Insert a piece of the copper wire (2.5 cm long) in the needle holder, and adjust the resistance so that the tip of the copper wire reaches a temperature at which the hormone paraffin-olive oil mixture begins to melt (±50 °C). In about 10 min, the temperature of the system reaches its working temperature, which remains very constant for long periods. In Figure 2 the construction of the apparatus is demonstrated.

Precautions. To avoid contamination, the needle must be changed after each sample of a dilution series. This can be done without cooling the apparatus by using a common pair of tongs. Another possibility is to glow the needle over a flame but we do not recommend this procedure.

When the current is switched on, the apparatus must always be hung in a rack, the needle down, otherwise the melted paraffin-hormone mixture which adheres to the needle will flow into the needle holder and will be a source of contamination.

Evaluation. With this apparatus, burns are completely avoided. The application of the hormone sample can be carried out much more quickly than with a needle which must be heated over a flame again and again.

Résumé. Une méthode rapide pour l'extraction des pupes de *Galleria mellonella* hors de leur cocons est décrite. Le schema d'un appareil très simple pour le test biologique d'hormones est donné; il évite les brulures de la cuticule pupale.

A. DE LOOF[4] and M. VAN DE VEIRE[5]

University of Ghent, Faculty of Agricultural Sciences, Coupure links 533, Ghent (Belgium),
27 September 1971.

[4] Dr. A. DE LOOF gratefully acknowledges a mandate as 'Aangesteld Navorser' of the N.F.W.O.

[5] This research was supported by the I.W.O.N.L. We wish to thank Dr. P. MOERMAN for his help in the construction of the apparatus.