localization may have on the genetic structure of populations and individuals. Such a mechanism would allow for the capture of co-adapted gene sequences which would not be continually disrupted by recombination within them. This may be of immense selective value in adapting local demes to the highly localized ecological conditions which they face, a characteristic of fossorial mammals in general and of pocket gophers in particular[4,6]. Secondly, it would allow for gene exchange between adjacent but karyotypically distinct populations as a means of inputting genetic variability into a population through selective introgression. Finally, it may allow for the maintanence of genic heterozygosity, even in small populations faced with strong inbreeding, a decidedly advantageous feature (see CARSON[7] for review). It has been shown elsewhere that, despite expected reduction in genic heterozygosity resulting from characteristics of the fossorial life mode, populations of *T. bottae* are as genically variable as other less restricted rodent species[8].

*Resumen.* El analisis de la primera divisiόn meiótica des roeder fosorial, *Thomomys bottae*, muestra una uniformidad notable en la colocación de quiasmas en los bivalentes, a pesar de las diferencias existentes en los cariotipos somáticos. La frecuencia promedio de quiasmas por bivalente es muy baja (1.03), y la mayoria de los bivalentes demuestran que sus quiasmas se localizan en o cerca de un estremo del par.

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## A Simple Technique for Obtaining Carbon Replicas from Small Organisms

Carbon replicas are widely used for surface studies. Depending on the properties of the subject to be replicated, different methods can be applied, which can be mainly divided into single- and two-stage methods[1].

The advantage of the single-stage method is the relatively small risk of artifacts. The two-stage method, on the other hand, is more laborious but has the advantage of leaving the surface structure undisturbed, thus enabling repeated study of the same surface.

In the single-stage method different techniques were employed to separate the carbon layer from the specimen. Unfortunately these techniques failed in the case of small organisms like aphids. The two-stage method also proved to be unsuccessful since the waxy surface structure is embedded and soluble in the replicating plastic, whereas a water-soluble plastic cannot be employed on account of the small dimensions.

To overcome the difficulties caused mainly by the small dimensions of the organism, a substratum which fixes the material was used.

*Method.* The procedure of replication can be divided into 6 steps: 1. A mixture of the powder and fluid of Technovit®/4071-d (KULZER & Co., 638 Bad Homburg, P.O. Box 261, West Germany) is prepared according to the directions for use. 2. A glass slide is covered with the mixture. 3. During the few minutes in which the plastic hardens, the organisms can be placed on it with a painting brush. Care must be taken to avoid contact of the surface to be replicated with the resin. 4. The dried preparation is now ready for carbon deposition (100 Å) in the vacuum plant. To prevent excessive shrinking the recommandations of Bradley, in Kay[1], concerning leaf replicas must be kept in mind: a reasonably short pumping time and a vacuum not much higher than $10^{-3}$ mm Hg. 5. To dry-strip the carbon film, a 4% solution of collodion in an anhydrous mixture containing equal parts of ether and ethyl alcohol is dripped on the assembly. The collodion must be dried at room temperature under conditions of low relative humidity. 6. The collodion-carbon film is then cut into small pieces, the collodion is subsequently dissolved in ether/alcohol, the replicas are mounted on grids (preferably 200 mesh/in, VECO, Holland) and shadowed if required.

The materials replicated in this study were abdomen and wing of the mealy plum aphid, *Hyalopterus pruni* (Geoff.) and leaves of the common reed, *Phragmites communis* Trin.

*Result and discussion.* The dry-stripping (stage 5) results in a collodion film with carbon only from the surfaces to be replicated and transparent on the places above the substratum, since the adhesive forces between Technovit and carbon are stronger than between carbon and collodion.

The carbon can be dry-stripped from an organism with a waxy surface layer by means of collodion, but it must be admitted that the wax and the carbon are not separated. In this procedure the wax is torn from the surface of the organism resulting in a collodion/carbon film encrusted with wax, a material which indeed is partly dissolved in the ether/alcohol mixture, but nevertheless contributes to an electron image with enhanced contrast. The adhesive properties of the substratum and the collodion 1. preclude ambiguity caused by replica picture of the Technovit, 2. eliminate an extra step of shadow casting which would be used only for the purposal of improving the picture, 3. but they make it impossible to obtain a replica from an organism of which the waxy layer is completely corroded, e.g., by mechanical or microbial action.

Another property of the collodion in which we were highly interested was the possibility of shrinkage. Therefore, we replicated leaf surfaces both in the usual way[2] and by employing the method described. As can be gathered from the photographs (Figures 1, 2), both techniques resulted in similar images. We thus concluded that the technique developed is satisfactory for the replication of small

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Fig. 1. Carbon replica of 1-week-old leaf of *Phragmites communis*, picked on 27th May 1971. The replica is obtained employing the method of JUNIPER and BRADLEY. The wax extrusions are examples of the two major types on leaves of common reed.

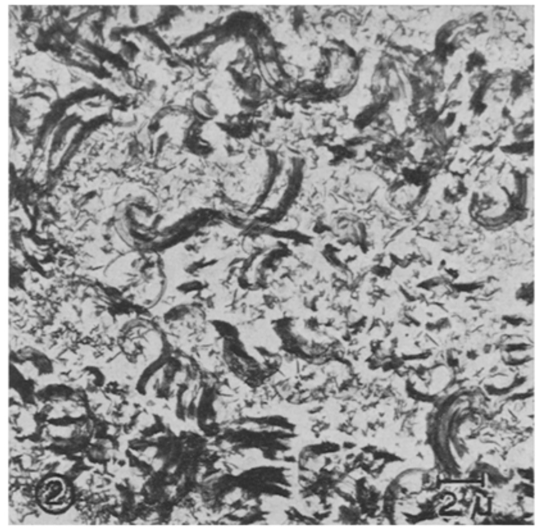


Fig. 2. Replica of a corresponding leaf, dry-stripped with collodion.

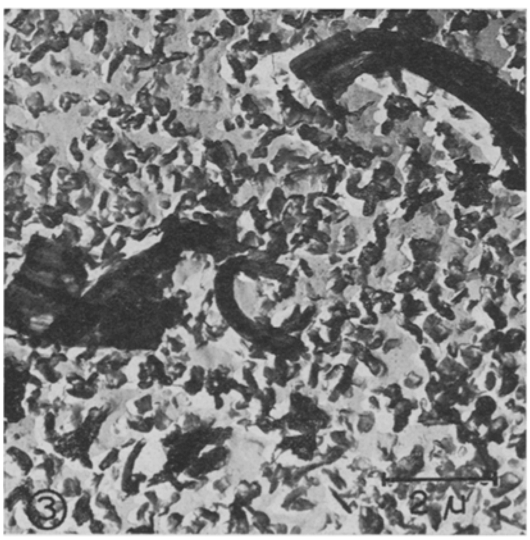


Fig. 3. Surface structure on the abdomen of Hyalopterus prunivirginoparae.


Fig. 4. Wing replica with the impression of a scale of the mealy plum aphid.

organisms like aphids (Figure 3). Therefore, this may be considered as a simple addition to the excellent method described by Juniper and Bradley[2] visualizing the ultrastructure of plant surfaces. Our technique combines reliability and simplicity so that even fragile objects such as aphid wings can be chosen for examination (Figure 4).

This technique may probably be quite useful for the expanding numbers of investigations dealing with the influences in the biosphere of pollutants[3–5] and biocides[6,7], which attack surface layers.

*Résumé*. Exposé d'une méthode permettant de faire des répliques au carbone de petits organismes tel que les aphides. L'utilisation d'un substrat de technovit® pour fixer les organismes est la partie importante de cette méthode. Le technovit a la propriété de coller la pellicule de carbonne; ainsi lorsque l'on détache celle-ci à l'aide de collodion, on obtient seulement la réplique se trouvant sur l'organisme.

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