gradual but rather rapid acclimation in corn? If such qualities do not arise in nature by or through gradual accumulation in association with constant crossing, how then did the first gene or unit of character arise? How did the first plant become resistant for any character?

I write simply to suggest that it is well for plant physiologists, ecologists and plant breeders to hold the open mind over against the thought of "Once a gene, always a gene."

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MECHANISM OF BUFFER ACTION IN SOILS

WHILE working on "The Rôle of Pectin in Jelly Formation" it was found that the buffer action of the pectin solutions was due entirely to the impurities in the solution and not to the colloidal properties of the pectin.

It previously had been assumed, while outlining the method for the attack of the problem involving a fundamental study of the mechanism of buffer action in soils, that the buffer action exhibited by certain types of soils was, for the most part, due to the colloidal content of the soils. This assumption was based on the fact that soils high in colloidal matter showed considerable buffer action while soils low in colloidal matter showed scarcely any buffer action.

In view of the results obtained with pectin solutions the plan of attacking the soils problem was changed so that now an attempt is being made to attribute the buffer action exhibited by the several soils to the impurities held by the colloidal fraction, perhaps by electrostatic attraction, double decomposition or neutralization of alkali with an acid or *vice versa*.

Some preliminary work has been done, using a Portsmouth loam, high in organic material. The colloidal fraction was separated and electro-dialyzed thus removing the greater part of the iron, aluminum, manganese, calcium, magnesium, sodium, potassium and other elements, as well as sulphates, phosphates and other acid radicles. As the electro-dialysis progressed samples were frequently withdrawn and their buffer action determined. It was found, during this preliminary work with this particular type of soil, that as the impurities were progressively removed from the colloidal organic fraction of the soil, the buffer action steadily decreased until, the impurities becoming negligible, the sample exhibited scarcely any buffer action.

As a result of this preliminary investigation the work is being continued, using various soil types with the hope of obtaining data sufficient to substantiate the claim that buffer action peculiar to soil types laden with colloidal material is not due directly to the colloidal properties of the soil but rather to the salts, metallic or acid radicles that are held by the colloidal fraction.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR OBTAINING INFECTIVE NEMATODE LARVAE FROM CULTURES[1]

CREEPING eruption, a human skin disease frequently encountered during the summer in some of the Southern areas of the United States, was shown by Kirby-Smith, Dove, and White[2] to be caused by third-stage nematode larvae. Later White and Dove[3] demonstrated that dogs and cats are concerned in the causation of the disease.

Much culturing has been necessary in the search for the adult worm of the causal parasite and in other studies in which infective larvae have been used. The useful Baermann apparatus was first employed to recover the infective larvae from the cultures. Later a still simpler method was devised which reduced very materially the time required. This latter method has been employed for a year and a half and has proved to be entirely adequate for the problem. An outline of it is given in the present article.

The method makes use of the fact, often observed, that the larvae of a number of parasitic nematodes as they approach the third larval stage and the close of the free-living period tend to migrate from the medium in which they are growing. The apparatus traps many of the migrating worms.

Convenient and sufficient equipment consists of crystallizing dishes 125 to 150 mm. in diameter, watch-glasses slightly larger than these dimensions respectively, Petri dishes 100 to 125 mm., test-tubes 20 by 150 mm., filter papers 9 to 12 cm., a spatula with a 4-inch blade, a test-tube rack, a three-quart boiler with cover, animal charcoal, and sterile water. Brief steaming in the covered vessel suffices for all sterilization that is needed.

The charcoal and the feces are properly mixed conveniently in one of the larger watch-glasses and transferred to the half of a Petri dish, with a moistened

[1] Read before the Washington Helminthological Society, April 16, 1927.

[2] Kirby-Smith, J. L., Dove, W. E., and White, G. F., "Creeping Eruption," *Arch. Dermat. and Syph.*, xiii, Feb., 1926, 137–173.

[3] White, G. F., and Dove, W. E., "Dogs and Cats Concerned in the Causation of Creeping Eruption." Official Record, U. S. Dept. Agr., Oct. 27, 1926, *V*.

filter paper covering the bottom. Sterile water is poured into a crystallizing dish sufficient to cover the bottom and into it is placed the half Petri dish containing the culture. A watch-glass is used as a cover. The apparatus (fig. 1) after labeling is placed for

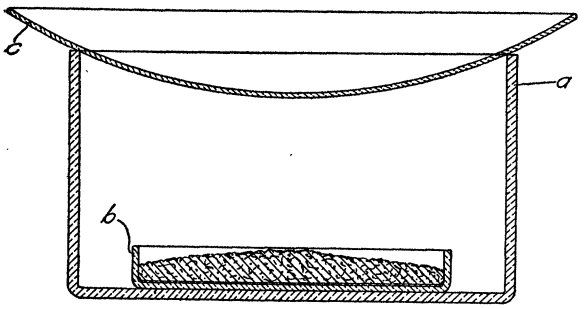

FIG. 1. Apparatus used for culturing nematode larvae. *a*, Crystallizing dish; *b*, Petri dish with charcoal-feces mixture; *c*, watch-glass cover. Water surrounds the Petri dish equal to about one half its depth.

incubation of the culture preferably where a high humidity can be maintained.

Many of the larvae on approaching the third larval stage migrate from the culture and are trapped in the water surrounding the Petri dish. In collecting them the watch-glass cover is removed and the half Petri dish with the charcoal culture is lifted out, preferably with forceps, and placed into it. The water containing the larvae is poured from the crystallizing dish into a test-tube which is then stood perpendicularly in the rack. The worms soon gravitate to the bottom of the tube, after which the water above may be pipetted off, leaving the larvae concentrated. The apparatus with the charcoal mixture may then be reassembled and sterilized by steaming.

A number of modifications of the apparatus and the method may be made to meet the worker's special needs. When there is but a small amount of culture it is well to use the half Petri dish with the bottom up. A Syracuse watch-glass or the top of a Coplin jar serves well in place of the Petri dish. Room temperature, especially in summer, may be substituted for the more constant one of an incubator.

A modified form of the apparatus has been used to reduce somewhat the amount of fungous growth in the culture when this seemed desirable. An aluminum pan of the diameter of the crystallizing dish, with the inclined side perforated, is placed beneath the watch-glass cover and supported by the edge of the dish. Into the pan is poured a few cubic centimeters of an aqueous solution of formalin. A 15 per cent. solution has been employed successfully, but the optimum strength should be determined by each worker to meet his own needs.

As many as 8 species of nematodes, representing 4 genera, have been cultured and isolated successfully by this method, an ample number of larvae of each species being obtained. In no case, however, have all the larvae in the culture been recovered. Additional ones may be had by transferring the charcoal culture to the Baermann apparatus.

While using the method one soon learns of its limitations and its advantages. The larvae are recovered from the cultures in relatively clean water. Only infective forms are obtained. A considerable leeway is permitted as to the time larvae may be collected from the apparatus. The first larvae trapped may be poured off, more water added, and the apparatus reassembled for later migrations. In field work in connection with studies on creeping eruption Dove and the writer have found this method for obtaining infective larvae very convenient and efficient.

In making studies on the migration of nematode larvae, Looss[4] trapped them in water but apparently did not employ the observation in devising a routine method for obtaining larvae from cultures.

Among those who have taken advantage of the migrating tendency of larvae in devising methods suitable for their studies is Darling,[5] who used Syracuse watch-glasses in the center of which he placed 3 to 5 cc. of stool and added sterile water until the feces were surrounded with fluid. The worms for study were taken from this margin of water. Fülleborn[6] also made use of this habit, employing agar plates. The charcoal-feces mixture was placed on this medium in the center of the Petri dish. The infective larvae migrating from the culture over the agar cause their trails to be inoculated with bacteria. By the growth of these the courses taken by the worms are readily observed. Sandground,[7] commenting on different culture methods, points out that the one employing the Baermann apparatus has served the needs of certain of his problems better than others which he has used.

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[4] Looss, A., "The Anatomy and Life-History of *Agchylostoma duodenale* Dub." *Recds. of Egypt. Govt. Sch. of Med.*, Cairo, 1911, *IV.*

[5] Darling, S. T., "Strongyloides Infectious in Man and Animals in the Isthmian Canal Zone," *Jr. Exp. Med.*, July, 1911, xiv, pp. 1–24.

[6] Fülleborn, F., "Nachweis von Ankylostomum durch Plattenkot-cultur." Vorl. Mitteilg. *Arch. f. Schiffs und Tropenhyg.*, 1921, pp. 121–123.

[7] Sandground, J. H., "Biological Studies on the Life-Cycle in the Genus *Strongyloides* Grassi, 1879," *Amer. Jr. of Hyg.*, May, 1926, vi, 337–388.