AGAMERMIS DECAUDATA COBB, STEINER, AND CHRISTIE; A NEMA PARASITE OF GRASSHOPPERS AND OTHER INSECTS.[1]

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INTRODUCTION

This is a preliminary account of a joint investigation carried on at Washington, D. C., and Falls Church, Va., and relates to a nema *Agamermis decaudata*, parasitic in insects, particularly in Orthoptera. The work at Falls Church is carried on with the cooperation of the Bureau of Entomology at its eastern field station, and all the insects examined have been determined by officers of that bureau. Other valuable assistance has been rendered by approximately 150 American zoologists and entomologists, whose aid has made possible a first tentative map of distribution of the parasite in the United States.

The general oversight of the work has been handled by the senior author, who has given special attention also to methods and apparatus, and to the structure and behavior of the free-living larva. Dr. Steiner gave special attention to the structure and taxonomy of the parasitic and post-parasitic stages. He has also handled nearly entirely the difficult matter of the literature and nomenclature. Prof. Christie has given special attention to field and laboratory work connected with the hosts and their habits, the effect on the host, the habits and distribution of the post-parasitic forms in the soil under natural conditions, and to the rearing of both host and parasite.

In the present account it is not proposed to do more than sketch some of the main features of the work, which has involved thousands of dissections, hundreds of carefully executed laboratory and rearing experiments, and the invention and trial of dozens of different pieces of special apparatus adapted to various features of the work.

A broad numerical basis has been given the facts disclosed, since the work has for its definite object the economic application of the data secured. The summary presents two aspects, the latter of which is subdivided: First, the results show positively that, in certain cases believed to be typical, the nema *Agamermis decaudata*, is an important factor in the birth rate of certain injurious insects; second, the application of this fact may be exploited in two ways, (*a*) by colonization—that is, moving the parasite from places where it exists to places where it does not exist, or is rare; the experiments show that this can be done, either by transferring diseased insects, such as grasshoppers, or by transferring the parasite itself in one form or another; (*b*) by forecast—since methods of detecting the parasite have been developed to a point where it is believed possible to hazard some form of prediction. Just as, if we can not control the weather, it is at least important to be able to predict it, so, if we can not prevent the ravages of an insect, it is at least important to be able to predict when the ravages are likely to occur. In this respect the results of the investigations are considered positive.

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SOURCES AND DETERMINATION OF THE NEMAS

In the main the material for these investigations was collected at Falls Church, Va., but questionnaires were sent to zoologists all over the United States. Nearly 160 of these were returned, some with collected Mermithid material, and many with very interesting information about distribution, hosts, etc.

The examination of this contributed material is not yet finished, and therefore no more than a somewhat brief sketch of the American Mermithid land-fauna can be given, together with some aspects of the economic value of the family.

On leaving the insect host the nemas enter the soil. There have been observed thus far a dozen or more species of Mermithids from American soil, mostly new. The two most common are what have been called *Mermis albicans* Von Siebold and *M. nigrescens* Dujardin, which are among the first Mermithids made known and also the ones most frequently mentioned in literature. Both apparently have a wide geographical distribution.

The determination of Mermithid species is difficult; one reason is that often only larval forms are obtainable, and another is that Mermithids do not possess obvious morphological characteristics. The present studies were much aided by the excellent papers of Meissner (*5*, *6*),[2] Rauther (*7*), and Hagmeier (*3*). The latter emphasized the opinion that among the principal and best aids in classifying Mermithids are the sense organs on the head, since they rarely change during the life of the individual.

Whereas *Mermis nigrescens* seems to show little variation and to be represented in this country by the typical European form, the American *Agamermis decaudata* is unusually difficult of determination, even for a Mermithid, because it presents an extensive series of variations, between the extremes of which there is a complete gradation of intermediate forms. These varieties, or races, and apparent hybrids are best recognized by the structure and arrangement of the head sense organs. As far as can be judged, the European *albicans* as described by Meissner (*5*, *6*), Rauther (*7*), and Hagmeier (*3*) has not yet been found in this country. The chaos of these forms is probably greatly augmented by hybridization. Cases occurred at Falls Church where the mating female and four males (four males with one female) all differed in the structure of the head sense organs.

Another interesting evidence of the existence of a number of different races in *Agamermis decaudata* is the fact that almost any collection that includes a number of female specimens will show intersexes—that is, females with more or less well developed male sexual organs. Experimental biology is showing that such intersexes may be frequent in the "artificial" crosses of closely related forms, races, or genotypes. *A. decaudata* as it occurs in nature, is not a clean-cut species, but rather a mixture of races and their hybrids.

The origin of the races of *Agamermis decaudata* is still uncertain. Perhaps the great variety of hosts may have some influence.

It may be considered questionable whether there are *decaudata* races special to certain hosts. We do not know yet, but it appears as if larvae of various races are entering hosts without special choice.

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[2] Reference is made by number (italic to "Literature cited," p. 926.

A review of the information obtained from the returned questionnaires also emphasizes the economic value of Mermithids, a fact surmised earlier by a number of entomologists but never proved in a substantial way. Of 130 returned questionnaires, 53.6 per cent state that Orthoptera were the hosts. In 51 cases the writers made observations regarding the effect of the parasite, mostly on an individual host. Of these latter observers, 85 per cent report an injurious effect on the host, 71 per cent stating that the normal development of the host was prevented, 47 per cent stating the fecundity of the host was reduced, and 53 per cent that the host was killed. Most of these statements seem mainly the result of fortuitous observation on a limited scale; in general, they corroborate those already mentioned in the literature. Hagmeier (*3*) estimated that on a meadow 33 per cent of all grasshoppers were infested with Mermis; Glaser and Wilcox (*2*) in this country estimated infestation up to 60 per cent; Leidy (*4*), very frequent; Steiner's observations (*8, p. 223*) showed in a garden heavily infested with the snail *Limax agrestis*, 20 to 30 per cent of them mermithized.

EXPERIMENTS AT THE EASTERN FIELD STATION OF THE BUREAU OF ENTOMOLOGY AT FALLS CHURCH, VA.

During the early part of July, 1922, it was noticed that grasshoppers in the vicinity of Falls Church, Va., were infested by a Mermithid parasite. From that time until cold weather, collections were made over an area of several square miles, and a total of 3,332 grasshoppers was examined. Of the 824 Tittigonidae included in this number, very nearly all were *Orchelimum vulgare* Harris. Of the remaining 2,508 Acrididae, about 90 per cent were *Melanoplus femur-rubrum* Deg. Mermithid infestation was found to average 12 per cent, increasing to about 25 per cent in heavily infested regions.

Records based on the examination of 2,498 grasshoppers, the sexes of which were determined before dissection, show the males and females to be about equally parasitized.

As the nemas do not reach sexual maturity in the host, but moult once in the soil after leaving the host, the determination of the material taken from grasshoppers is a difficult task. However, it is certain that the greater number of the nemas were varieties or races of *Agamermis decaudata*, with an occasional species of another kind.

The parasites begin to leave the hosts by the latter part of August, if not before, and are nearly all out by the first of October. Their method of exit is by forcing their way through the body wall of the insect. In one carefully observed case the parasite issued from the side of the abdomen, head foremost, and was free in a very few minutes. The parasite was assisted by the kicking of the grasshopper, whose feet became entangled in the coils of the nema.

Once free from the host, the Mermithids make their way into the soil to a depth of 3 to 20 cm. The females go down from 4 to 8 cm., seldom deeper. Here they coil up in the "nest," forming a "knot," and apparently never again move about. The males move through the soil more freely, as it is evidently their function to seek out the females. The "knots" as dug from the soil during the winter contain one female, rarely more, and from one to seven males.

In order to determine the approximate Mermithid population in an infested region, 16 square feet of soil were examined to a depth of 30 cm. (12 in.). The yield was 132 Mermithids, or at the rate of 359,370 per acre.

Eggs are laid in large numbers until both the nemas and the wall of the nest become plastered with them. Egg counts show that each female lays, on an average, at least 5,000 eggs. Under the 16 square feet referred to in the preceding paragraph, 32 "knots" were surrounded with eggs. If the average number of eggs per nest is 5,000 (probably a very conservative figure) there will be, provided all the eggs hatch, 435,600,000 larvae per acre. Each foot will therefore support a population of 10,000, and each square inch of surface will have below it about 70 of these young Mermithids

The egg has the shape of a slightly flattened sphere, and measures 119 by 105 microns. The rate of its development is influenced by external conditions, particularly temperature. At the end of five or six days, with average September weather, the embryo takes the form of a single, circular coil, almost filling the shell. At this point in its development there is a rapid proliferation of cells at the posterior extremity, the ends of the loop overlap and in a short time several coils are developed. As the length of the embryo increases new coils are formed until, at the end of about a month, the young larva reaches its full length of a dozen or more coils—that is, 3 to 4 mm. The development of eggs deposited late in the fall seems to be retarded by cold weather, for material collected in March still contains the earlier stages of development. The hatching of the eggs is probably brought about by the warm spring weather, as eggs brought into the laboratory in winter and kept at room temperature hatch freely after three or four days.

After hatching, the larvae work their way to the surface of the soil and enter the newly hatched grasshopper nymphs.

Artificially infested grasshoppers containing six or eight Mermithids die in about eight days. In fact it may be doubtful if grasshoppers harboring more than one parasite ever reach maturity. The ovaries of infested females which do survive are vestigial, never producing functional eggs, and it is likely that the males are also rendered sterile. The exit of the nemas, sometimes at least, results in the death of the grasshopper, and there is every reason to believe that this is always the case.

STRUCTURE AND BEHAVIOR OF THE FREE-LIVING LARVA

The rear five-sixths of the *decaudata* larva has a certain uniformity of structure. Here the cuticle and body wall are typically nemic. The body cavity is filled by a moniliform series of elements, having somewhat the general character of nemic intestinal cells, but there is no lumen and no trace of an anus. Each of these 50 to 70 cells (trophocytes), in addition to its nucleus, protoplasmic network, and minute proteid granules, is packed with spherical 3–4 micron fat globules, melting at about 55° C., and contains a thin-walled catabolic vesicle holding a birefringent crystal. As the larva ages, the fat disappears, while the vesicle increases, thus pushing all other contents of the cell to one end. In a few weeks no fat remains, and the nema has a segmented aspect. By expending the fat of the trophocytes, the posterior five-sixths of the nema becomes the active mechanism for driving the strongly cephalated anterior sixth to, and into, the host.

On entering the host the driving mechanism is shed at a predetermined, elaborately prepared node, and only the cephalic portion becomes parasitic. The node at which this automatic amputation takes place is prominent in the free-living larva. Persisting as a little altered terminal scar, it indicates that no moult occurs during the parasitic life. Usually the driving mechanism is left outside the host, but young larvae in which the node is unripe may take the driving mechanism inside, where, in the course of a few days, it is shed. The nema requires from two to five minutes to enter the host. This period is often preceded by a few minutes of waiting—perhaps devoted to some internal preparation for the onslaught. Entrance is bored at any thin part of the cuticle of the very young larva of the host—namely, on the head, thorax, abdomen, or legs.

The cephalic end of the free-living larva is highly specialized. No particular reference is made here to its acute, hollow, protrusile spear, or its six inconspicuous cephalic papillae, amphids, nerve-ring, oesophageal lumen, and longitudinal chords,[3] most of which closely resemble those of the "*Mermis albicans*" of Hagmeier. The bifurcated, symmetrical, two-celled renette lies in the neck behind the nerve-ring. The oesophagus ends posteriorly in a short, small, cylindroid swelling, which at intervals of about a second may exhibit the sudden peristaltic motions characteristic of the swallowing act of nemas.

The intestine comprises two sections, of which the posterior is about two bodywidths long. The four times longer anterior section, comprising sixteen cells, has a distinct, narrow, refractive, tubular lumen, and is flanked by three unequal glandular structures having their free ends caudad, the largest of which, the right, extends throughout the length of this section of the intestine, while the two others—equal, narrower, and on the left—have only half that length. These organs are packed with minute dodecahedral, colorless, in the main nonstaining (carmine, osmic), refractive granules, of which those in the largest organ are very much the largest; beside this, there are physiological differences, in that the two smaller organs stain differently intra vitam from the large one and behave differently after the larva enters its host. During the early days of parasitism these three organs disappear, so they evidently have to do with the physiological changes connected with the inception of taking in food from the host. The intestinal lumen in the 20–30-celled posterior section is of a different, nonrefractive character (wider and surrounded by a very thin nonrefractive wall), and this section presents the elements commonly seen in the intestinal cells of free-living nemas.

The sexual blastomeres nearly always lie in the axil between the two portions of the intestine.

After entering the host the posterior portion of the intestine changes and gives rise to a syncytiumlike (?) structure of relatively enormous size. As the three glandular organs disappear, the anterior 16-celled section of the intestine also begins a relatively huge growth, at first (20 days) forming a somewhat moniliform series of 16 organs (urocytes?) with large nuclei very like the four organs of *Tetradonema* (*1*). The early growth of the two portions of the intestine is not so very unequal, so that the sexual anlage comes to lie nearer the middle of the body. Along with this enormous growth of the intestinal elements the longitudinal chords,[3] especially the lateral, increase greatly in volume from back to front.

In spite of the fact that the free-living *decaudata* larvae will live for hours or even days on thawing ice, when placed in water or moist earth at 32° F. they are much injured by the congealment. Two or three such experiences kill them. One exposure to zero Fahrenheit kills them. It seems probable that they can not survive even light frosts in nature.

[3] Hitherto usually referred to in the literature as the "longitudinal fields."

These free-living larvae can exist in soil for several months, during which time experiments have shown them to be apogeotropic and positively phototropic, rheotropic, pedotropic,[4] and xenotropic.[5] Within the limits of the vernal temperatures of arable soils in subtemperate regions, the larvae are positively thermotropic. Once dried they die.

From what has been previously said concerning the deposition of the eggs, it will be seen that these tropisms tend to bring the larva to the surface of the ground and into contact with its host.

The entrance of the larva into its host, although very easily brought about in the laboratory, has not been observed in nature and doubtless will be a matter very difficult of observation. The forecast is that the larvae will come to the surface of the ground in late spring, leave the soil, lurk among the decayed and living vegetation near the surface of the ground, and enter the host mostly at night.

The bobtailed parasitic larva, 25 by 700 microns, grows at a rapid rate, often increasing a millionfold in two to three months.

BRIEF GENERIC AND SPECIFIC DIAGNOSES[6]

Agamermis Cobb, Steiner, and Christie n. gen.

Mermithidae with terminal mouth, no mouth papillae, 6 head papillae, vagina S-shaped, 2 spicula; parasitic larva decaudate. Moults once in the egg.

Agamermis decaudata Cobb, Steiner, and Christie n. sp.

Characters as given on pages 924–926 herein, and as shown by Hagmeier's figures (except fig. 14) for his *Mermis albicans* (3). Amphids, however, varying somewhat from those figured by Hagmeier; no cervical papillae seen. Moults once in the egg.

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[4] πέδον=soil.
[5] ξένος=host.
[6] Full descriptions will be published later.