PROTOZOAN PARASITES OF THE ORTHOPTERA, WITH SPECIAL REFERENCE TO THOSE OF OHIO[1]

I. Introduction and Methods

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INTRODUCTION

This work is primarily a distributional and ecological study of the protozoan parasites of Ohio Orthoptera, exclusive of the domestic cockroaches, *Blattella germanica*, *Blatta orientalis*, and *Periplaneta americana;* and also excluding the wood-eating roach, *Cryptocercus punctulatus*. The Orthoptera were taken from July, 1932 to October, 1933 in Franklin County and the six contiguous counties of central Ohio, and in the southeastern portion of Washington County in southeastern Ohio; also sporadically in four other counties of Ohio and the Lake Michigan sand dunes of Indiana.

Specifically, the purpose of this study was to determine the distribution of protozoan parasites in all available species of Orthoptera in a given region, and to learn something of the relationship between life history and ecology of parasite and life history and ecology of host. In addition, a classified, annotated list of the protozoan parasites of the Orthoptera of the world was compiled from the literature, a key for the identification of these protozoa was constructed, and a complete bibliography was assembled.

A comprehensive survey of the protozoan parasites of all the Orthoptera of a given geographic region has not previously been made, although S. F. Bush has published (1928) such a study of the Gregarinida present in the Acrididae (locusts or short-horned grasshoppers) of a region in South Africa.

The terminology of this work has been selected, for the most part, from Calkins, 1933 (general protozoology), Wenyon, 1926 and Kudo, 1931 (parasitic protozoology), and Watson, 1916, p. 10 (Gregarinida).

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Professor W. J. Kostir, of the Department of Zoology and Entomology, The Ohio State University, suggested the possibilities in this field and kindly undertook the supervision of the research. Mr. Edward S. Thomas, curator of Natural History of the Ohio State Museum, helped greatly in many ways, particularly in the collecting and identifying of the Orthoptera. Dr. Theodore H. Hubbell, of the Zoology Museum, University of Michigan, generously devoted much time to the correction and revision of the classified list of orthopteran hosts examined by other investigators. The following persons also rendered valuable help: Dr. A. N. Caudell, Entomologist, United States National Museum; Dr. Richard R. Kudo, Department of Zoology, University of Illinois; Mrs. Ethel M. Miller, Botany and Zoology Librarian, The Ohio State University; Professor Catherine B. Semans, Youngstown College; and Dr. B. P. Uvarov, British Museum of Natural History.

METHODS

Orthoptera were collected extensively throughout central Ohio and a portion of Washington County, and less so, in several other localities, with a view to obtaining as large a number of species from as great a variety of habitats as possible. There was no thought of collecting at regular intervals from the same localities, as that was not possible in the time available. The majority of the collections were made during the summer and early fall of 1933; hence no attempt was made to compare results from the same seasons (summer and fall) of the two different years; and, also, the number of specimens of no one species was great enough to justify such a procedure. Most of the collecting was done by net sweeping, although all known methods were used.

The following ecological factors were recorded for each locality in which collections were made: Uppermost geological stratum, kind of soil, topography, average soil moisture and soil pH, plant association, and the general weather conditions. The pH was determined by the use of the Soiltex colorimetric method—a simple modification of that of M. F. Morgan (1927, p. 387; also described by E. F. Snyder, 1928, p. 18)—supplemented by the Hellige colorimetric method. In the Soiltex method, a small portion of pulverized soil is placed in a trough of waxed paper which is held on a slant. Several drops of indicator solution are dropped on the upper end of the soil

until a globule of liquid appears at the lower end. A small, clear drop of the liquid is drawn away from the soil with a clean knife blade and its color is compared with a color chart. An average discrepancy of 0.32 occurred between determinations made by this method and the quinhydrone electrometric method on four samples of soil covering a range of 5.58 to 7.71. The error was, no doubt, partly the result of the presence of pigments in the soil. The same method was used in determining the pH of the anterior end of the mid-intestine of thirty-seven orthopteran specimens, in an attempt to correlate parasite prevalence with degree of acidity.

The general weather data are omitted from this paper because these were found to be of little significance over so comparatively short a period of collecting.

The captured insects were kept alive in glass jars, usually containing pieces of paper or dry plant material to reduce the humidity—apparently the greatest factor in the loss of insect material—and also to prevent the insects from striking one another. A rapid change of temperature was even more deleterious than high humidity, but it did not occur so often. The examination was usually made immediately after taking the insects, hence, except in special cases, no attempt was made to provide them with food.

In preparation for the examination of insects for parasites, a thorough study of insect morphology was made, including the histology of the alimentary tract of the differential locust (*Melanoplus differentialis* (Thomas, 1865) Brunner, 1885).

The perfected routine as used in the examination of the insects may be resolved into the following steps:

1. As a preliminary step the following information was recorded: Accession number of the insect; age and sex; the locality (characterized ecologically in a field record-book) where the insect was taken; the dates of capture and examination; and, later, the parts examined and the percentage of saline (0.85% sodium chloride) solution used in the temporary mounting. An experiment on the effect of three percentages of saline (0.65%, 0.75%, 0.85%) and distilled water on a delicate gregarinid (*Gregarina galliveri* Watson) which was available in comparatively large numbers, showed that there was little appreciable difference, except with the distilled water, where distortion (rounding) was produced somewhat earlier.

2. The insect was slit anteriorly on the midventral line with a small pair of scissors. In rare cases where the insects were extremely small and active, they were anesthetized with tobacco smoke. The severing of the ventral nerve trunk in the thoracic region proved to be no more effective in stunning the insect than the longitudinal slitting.

3. The cut edges were pushed well apart with a pair of forceps and, with a second pair, the alimentary tract was broken from its extreme connections and removed to a clean glass slide into a few drops of saline solution. (See step 11 for the method of examining the intestinal contents of cockroaches from flagellates and ciliates.)

4. The insect was pinned and labelled.

5. The slide mount was placed on the stage of a low-power (×10.5 to ×30) binocular microscope. The alimentary tract was broken apart by means of two pairs of forceps—cleaning them after each operation—into fore-intestine, mid-intestine (removing the enteric ceca for separate consideration), and hind-intestine—or whatever divisions of the latter were present. Each section was placed in a separate drop of saline solution.

6. The alimentary tract and malpighian tubules were examined externally for celomic parasites, and then, with two stout insect pins (cleaned after each operation), each section was torn into small shreds, the number and position of the larger Gregarinida being noted. The malpighian tubules were left more or less intact for separate examination.

7. The drops of saline solution impregnated with gut contents were spread over the slide to make them as thin as possible and were examined—usually without a cover-glass, the use of the latter making it difficult to obtain organisms for permanent staining—with the ×60–100 combination of the microscope, stepping up the magnification as high as ×880 for minute flagellates. The examination was facilitated by the use of a graduated mechanical stage.

8. In a thorough examination of the temporary mount, everything of apparent significance was recorded, measurements of the organisms being determined by means of a calibrated ocular micrometer. Drawings were made with or without the use of a camera lucida, depending upon the amount of movement in the organisms. Weak stains were used at the start but were abandoned in favor of the saline mount followed by the permanent staining of all representative parasites, including helminths.

9. Cysts of interest, if not permanently mounted, were put in a watch glass or the well of a concavity slide and kept in a moist chamber, but a technique for invariably bringing about cyst dehiscence was not perfected, as the number of cysts was too small.

10. Representative gregarinids (i. e., of the order Gregarinida) were removed, by means of a capillary pipette, to a watch glass for fixing and staining. It was found to be impracticable to make permanent mounts of flagellates from saline, as it was difficult to make them adhere to glass.

11. The fixing and staining of flagellates and ciliates (from step 3): A portion of the hind-intestine—usually the colon—of cockroaches was mixed with a small drop of sterile horse serum (adhering agent) on a clean cover glass, spread over the entire surface of the latter, and plunged into a fixing agent (preferably Kahle's fixing fluid: 32%, 95% ethyl alcohol; 11%, 40% formaldehyde; 4% glacial acetic acid; 53% distilled water) in a syracuse watch glass for about two hours;

then it was passed through 70% and 50% alcohol down to 30%, at fifteen-minute intervals, and stained ten to fifteen minutes by Ehrlich's hematoxylin (not longer, as in the case of other hematoxylin methods, otherwise overstaining results), and back up through the same alcohols to absolute alcohol and then xylol for the mounting in balsam.

12. The fixing and staining of Gregarinida (from step 10): Instead of using adhering agents, which created an undesirable background when stained (except for the smallest specimens), the saline was removed and the fixing agent (preferably Kahle's) applied by means of a capillary pipette—the method used throughout the entire fixing and staining procedure. Kahle's fluid was left about two hours, followed by two changes of 70% alcohol (two hours each), 95% alcohol (two minutes), the stain, Fast Green FCF (two minutes), 95% alcohol again (one minute), absolute alcohol (five minutes). The organism was placed by means of a capillary pipette directly into balsam (mixed with xylol) on a slide cleaned with xylol and a clean cover-glass was applied.

The Kahle's-Fast Green method is a slight modification of that suggested by C. H. Kennedy (1932) for the staining of the cytoplasm of the insect cell, and was discovered to be effective for the Gregarinida through the staining of sections of the alimentary tract of *Melanoplus differentialis* (Thomas) containing sporonts of *Gregarina rigida* (Hall). No additional nuclear stain is necessary, as the refractive index is quite different from that of the cytoplasm. Several other staining methods were tried, but none appeared to be as satisfactory as that described above.

All permanent slide mounts were placed in the author's private collection.

13. Every insect examined, irrespective of whether or not it was a host, was taken to Mr. Edward S. Thomas for identification. Many of the specimens were retained for the Ohio State Museum collection and were so labelled that they may be readily referred to. The remaining specimens (all of the hosts and many of the non-hosts) were placed in the author's private collection.

14. The permanent slide mounts were examined, and everything of significance was recorded, this information being compared with that acquired from the examination of the temporary mounts. The biometric method of M. E. Watson (1916, p. 42), modified, was adopted for the description of the Gregarinida. At least one representative gregarinid individual from each host individual was described in detail. The position of the nucleus was recorded, but as that structure may shift its position somewhat, as was observed in at least one case, it is doubtful that the information will prove to be of much significance. Hence, it will be withheld for further consideration.

TERMINOLOGY OF THE ALIMENTARY TRACT

Because of the confusion existing in the use of terms describing the divisions of the alimentary tract of Orthoptera, it is well to consider them at this time.

The embryological divisions of the alimentary canal are

the fore-, mid-, and hind-intestine. In the adult fore-intestine ("fore-gut"), the following parts, from the anterior to the posterior end, are recognized: Salivary glands—anterior evaginations, rudimentary or lacking in the Acrididae; esophagus; crop—an esophageal expansion; and gizzard ("proventriculus"), rudimentary in the Mantidae, almost vestigal in the Phasmidae, and vestigal or wanting in the Acrididae. The mid-intestine ("mid-gut," "ventriculus," "chylific stomach") consists of the enteric ("gastric," "pyloric") ceca—anterior evaginations, and the mid-intestine proper; the number of enteric ceca in the various families is as follows: Blattidae and Mantidae, eight; Phasmidae, none; Acrididae, six; and the Tettigoniidae, Gryllacrididae, Gryllidae, two—these being sac-like, while those of the other families are more tubular. The hind-intestine ("hind-gut") consists of the malpighian tubules—anterior evaginations variously grouped in the different families; small intestine—only in certain Blattidae; large intestine ("colon"); and the rectum—in many, little more than a colic expansion.

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