

ARTICLES

SOME RELATIONSHIPS BETWEEN BACTERIA AND CERTAIN SEWAGE-INHABITING INSECTS¹

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The larvae of a number of dipterous insects breed in sewage-polluted waters. There is an evident possibility that at least some of these larvae may acquire disease-producing organisms that may later be disseminated by the adult flies which often occur in enormous numbers in cities adjacent to polluted streams and lakes. In 1942 we had the opportunity to test the tenability of this hypothesis. The data obtained during this study have been briefly referred to in a previous publication (Steinhaus, 1946), but have never been presented in detail. In light of the recent report by Lysenko (1957) on this same general subject—but with different results—it would seem appropriate to present here at least a summarization of the results obtained and the conclusions reached.

The insects selected for this investigation were *Tendipes decorus* (Joh.) (= *Chironomus cayugae* Joh.) *Tendipes plumosus* var. *ferrugineovittatus* Zett., and *Psychoda* sp. The *Tendipes* species normally breed in the bottom mud of lakes and streams, and the adults are found in large numbers near water. The psychodid used in these experiments breeds in large numbers in trickling filters and the adults appear in swarms at the time of emergence. The larvae were collected in the field, placed in sewage-contaminated mud, and subsequently examined in the laboratory.

¹ This study was made in 1942 when the senior author was a member of the staff of the Rocky Mountain Laboratory (Hamilton, Montana), and the junior author was on the staff of the Stream Pollution Investigations Laboratory (Cincinnati, Ohio). Both laboratories are units of the National Institutes of Health (USPHS).

STUDIES ON *Tendipes* spp. A total of 36 larval and 58 adult *Tendipes* were studied.

Larvae. The results of the microbiological examinations of the larvae are given in Table I. Of 16 specimens examined, taken directly from sewage-polluted water, the body contents of 8 yielded large numbers of bacteria of which 11 strains were isolated. In order to determine whether these 11 strains were regularly and normally present in the larvae or had been ingested, a number of larvae which had been developing in sewage-contaminated mud were removed, washed several times and placed in several changes of a filter paper suspension over a period of 4 or 5 days. The body contents of 13 of 21 specimens fed on filter paper suspensions yielded 17 strains of bacteria. Nearly every one of the 13 harbored internally a gram-negative small rod which was abundant in the filter paper suspension itself. They apparently lost those bacteria that may have been originally present in their alimentary tracts. This observation indicates that the bacterial flora very probably originates in the substrate.

From these data it may be concluded that a considerable number of *Tendipes* larvae living in sewage harbor bacteria. The flora consists chiefly of gram-negative small rods (mostly coliforms, as would be expected from a sewage habitat), although gram-positive cocci and rods (including anaerobes) were likewise found. Diatoms and other algae were also present occasionally.

In a similar investigation of *Tendipes*

TABLE 1.—Bacterial flora of *Tendipes* larvae
Larvae from Sewage and Sewage-Contaminated Mud

Method of Examination	Number of Specimens Examined	Number Yielding Bacteria	Number of Bacteria Isolated	Kinds of Bacteria
Triturated	12	7	10	8 gram-negative rods 1 gram-positive coccus 1 gram-positive anaerobe
Dissected (alimentary tract)	4	1	1	1 gram-negative rod
Totals	16	8	11	9 gram-negative rods 1 gram-positive coccus 1 gram-positive anaerobe
Larvae Fed on Filter Paper Suspension				
Triturated	17	10	14	11 gram-negative rods 1 gram-positive coccus 1 gram-positive small rod 1 gram-positive anaerobe
Dissected (alimentary tract)	4	3	3	2 gram-negative rods 1 gram-positive short rod
Totals	21	13	17	13 gram-negative rods 1 gram-positive coccus 2 gram-positive small rods 1 gram-positive anaerobe

(probably *dorsalis*) larvae, Hurley (1951) found the flora to consist of approximately 41 percent gram-negative rods, 33 percent gram-positive rods, and 25 percent gram-positive cocci. These same organisms were shown to be present in the environment of the larvae. Although we made no determinations along such lines, Bonnell and Mote (1941) state that *Tendipes* larvae may possibly use bacteria as food.

Adults. Table 2 gives the data pertaining to the emerged adults examined immediately after leaving sewage-contaminated mud and water. The procedures used excluded the possibility of secondary contamination, since aseptic techniques were employed throughout the handling and examination of the insects. Fourteen of 31 triturated specimens examined yielded abundant bacteria (22 strains); there appeared to be little difference in the bacterial flora of the males and females; about one-third of the specimens of each har-

bored bacteria. As in the case of the larvae, the majority of the bacteria consisted of gram-negative rods (17 out of 22 strains). Three cocci, a gram-positive small rod, and a gram-positive anaerobe were also isolated.

EXAMINATION OF INTESTINAL CONTENTS OF ADULTS. Soon after emerging, the adults frequently released small deposits of greenish intestinal fluid (meconia). For the purpose of determining if the bacteria were present in this fluid several adults were carefully and aseptically placed on solid media in petri dishes as soon as possible after emergence. After a few hours one could often find several deposits on the media. The midges were then removed and the plates incubated. The results of this experiment are given in Table 3. None of five males yielded bacteria. Four of 7 females yielded large numbers of 4 strains. These were gram-negative small rods.

TABLE 2.—Bacterial flora of adults (triturated) emerged from sewage

Sex	Number of Specimens Examined	Number Yielding Bacteria	Number of Bacteria Isolated	Kinds of Bacteria
Males	7	2	2	1 gram-negative rod 1 gram-positive small rod
Females	8	3	3	2 gram-negative rods 1 gram-positive coccus
Undetermined	16	9	17	14 gram-negative rods 2 gram-positive cocci 1 gram-positive anaerobe
Totals	31	14	22	17 gram-negative rods 3 gram-positive cocci 1 gram-positive small rod 1 gram-positive anaerobe

The following additional facts are of interest: Five of the specimens (4 males and 1 female) yielded no bacteria in their meconia, and none when triturated and cultured. One female and one male (the latter from the *Serratia marcescens* experiment which is discussed later) yielded no bacteria in their meconia but did when triturated and cultured. Another female yielded a gram-negative rod in its intestinal deposits and the same bacterium when the specimen was triturated. One male (from *S. marcescens* experiment) yielded a gram-negative rod in its intestinal deposits but none when triturated and cultured. Thus it may be seen that the intestinal contents deposited soon after emerging may or may not be sterile. Twelve of 18 that were allowed to walk on plates of solid media contaminated the plates, while 6 did not.

Of interest is the fact that Hurley (1951) has also found that the fecal material of *Tendipes* adults contains the same

kinds of bacteria found in the water they inhabited during the larval stage.

EXPERIMENTS WITH *Serratia marcescens*. In an effort to trace a known organism from the sewage to the emerged adult, a 24-hour broth culture of the red-pigmented bacterium, *Serratia marcescens* Bizio, was added to the sewage in which were living the *Tendipes* larvae. When the adults emerged, they were examined for *S. marcescens* by trituration and by examination of their intestinal deposits. Only 2 of the 12 triturated adults yielded *S. marcescens* (one male and one female). Neither of these yielded *S. marcescens* when it walked over the surface of a solid medium and the bacteria were not found in the intestinal deposits. One other adult did yield *S. marcescens* when it walked over a plated medium, though when triturated this specimen was negative. No *S. marcescens* was isolated from its intestinal deposits.

KINDS OF BACTERIA ISOLATED. Since the

TABLE 3.—Bacteria in intestinal deposits of adults emerged from sewage-fed larvae

Sex	Number of Specimens Making Deposits	Number in Which Deposits Negative	Number in Which Deposits Positive	Kinds of Bacteria
Males	5	5	0	None
Females	7	3	4	4 gram-negative rods
Totals	12	8	4	4 gram-negative rods

insects studied in this investigation did not appear to possess a characteristic bacterial flora of their own, detailed identifications of the various strains isolated were not carried out in all cases. As was to be expected, most of them were coliforms. The 71 strains isolated consisted of the following morphological types:

- 56 gram-negative small rods
- 3 gram-positive small rods
- 7 gram-positive cocci
- 2 gram-positive spore-forming aerobes
- 3 gram-positive spore-forming anaerobes

STUDIES ON *Psychoda* sp. Nineteen strains of bacteria and 1 fungus were isolated from the body contents of 18 specimens (8 adults and 10 larvae) of *Psychoda* sp. From the 4 adults, 6 strains of bacteria (2 gram-positive cocci, 3 gram-negative rods, and 1 gram-positive small rod) were isolated. The other 4 specimens were sterile. From the 10 larvae, 13 strains of bacteria (all gram-negative small rods) and 1 fungus were isolated. None of the larvae was sterile.

DISCUSSION AND SUMMARY. Fifty to one hundred percent of the individual specimens of several lots of the larvae of the *Tendipes* and *Psychoda* studied were found to contain bacteria. Forty-five to fifty percent of the adults were shown to harbor the same types of bacteria as found in the larvae. Most of the bacteria isolated from both the adults and the larvae were coliforms (79 percent). The larvae fed on filter paper suspensions were found to contain the same types of bacteria as found in the suspensions themselves, and they harbored as many bacteria as the larvae feeding on sewage.

Approximately 33 percent of the adults

releasing the initial intestinal fluid ordinarily exuded after emergence, deposited bacteria, nearly all of which were coliforms.

One may assume, therefore, that the adults' emergence from sewage can spread sewage bacteria by their intestinal deposits. They might also spread bacteria by contact since 66 percent of the adults that walked over the plates yielded bacteria. Where these midges occur in enormous numbers they cause considerable loss and annoyance by getting into food and other materials used by people.

The results obtained in the experiments recorded here indicate that insects, such as species of *Tendipes* and *Psychoda* that may live as larvae in sewage, are capable of acquiring microorganisms during the larval stage and later of disseminating them during the adult stage. This conclusion is confirmed by data obtained in a subsequent but similar study by Hurley (1951). The fact that Lysenko (1957) found that certain test bacteria did not accompany *Tendipes plumosus* throughout its metamorphosis may possibly be explained on differences in the microorganisms and testing procedures used.

References

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Be sure to plan ahead for the AMCA meetings and make your hotel and travel reservations in plenty of time. Remember, February 22 is a holiday and it may be difficult to arrange for travel the last minute.