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The Breeding System and Distribution of *Tetrahymena pyriformis.**

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SUMMARY. Seven hundred twenty samples of water from 46 states and the province of Ontario, Canada, were collected and examined for *Tetrahymena pyriformis*. Thirty-four hundred clonal cultures of the ciliate were isolated from the 154 (21%) positive samples and grown axenically in peptone medium.

The breeding behavior of strains from these 154 sources was investigated by mixing clonal cultures in various combinations. Eight sources yielded selfing strains and 52 yielded strains capable of mating only in certain combinations. Thirty new mating types in seven new non-interbreeding varieties were discovered. Ninety of the 154 collections yielded non-conjugating strains. Fifty of these proved to be amicronucleate.

Distribution of variety 2 organisms was the most widespread; 22 collections from 11 states and Ontario, Canada, were obtained. Variety 3 was collected 14 times from seven states. Other varieties were found less often. The ciliate was found about as often in running as in standing water, but certain varieties were found predominantly in one or the other habitat.

THE DISCOVERY of sexuality(10,6) and of mating types(3) in the protozoön, *Tetrahymena pyriformis*, opened new areas of investigation of this organism. Previously this ciliate had been extensively used in studies of animal nutrition and metabolism, but no studies in genetics had been possible. In view of the present status of knowledge regarding protozoan genetics and the extensive information on the biochemistry of *T. pyriformis*, genetic studies on this ciliate could be significant.

Before certain breeding programs could be undertaken diverse experimental stocks were needed, and the breeding behavior of this ciliate needed elucidation. Attention was focused on these needs when it was considered that all of the original breeding stocks, except strain WH52(3,4,13), were derived directly or indirectly from one pond in the vicinity of Woods Hole, Massachusetts; and that 100 other clones isolated from several nearby habitats could not be induced to mate in any of the trial mixtures(3). In hopes of finding other varieties and mating types an investigation was begun, and this paper deals primarily with the breeding system and geographic distribution of the new varieties and mating types discovered.

MATERIALS AND METHODS

Water samples from many diverse habitats in the United States were collected and examined for the presence of *T. pyriformis*. Collections made during automobile trips were in 8-ounce jars but smaller ones were more convenient for mailing samples requested from persons in distant localities. Water samples were collected every month of the year during the period from the summer of 1952 to the winter of 1954-55. Those obtained during long collecting trips in summer were kept cool until they reached the laboratory. Samples in jars or finger bowls were fed a surface sprinkling of Bacto-tryptone, were incubated at room temperature and were periodically examined. When *T. pyriformis* was present, 20 to 30 clonal cultures were axenically established in peptone medium(4) through the use of 250 μg./ml. each of penicillin G and streptomycin in culture medium. For comparative purposes organisms from several collections were isolated directly into bacterized lettuce medium without being exposed to the antibiotics. Final identification was made through the use of the Chatton-Lwoff technic of silver impregnation(2). The identification of all new varieties reported herein was confirmed by Corliss (personal communication).

Forty-one sexually non-reactive clones, collected during the summer of 1952(3), were on hand at the beginning of this study. These were designated UM1-41. In addition to these, representatives of the seven previously established mating types were obtained from A. M. Elliott (I-III) and D. L. Nanney (IV-VII), University of Michigan. All other strains included in this report were isolated from nature during the study. These have been assigned the letters UM with a numeral designation, in keeping with current practice.

Experimental stocks were subcultured frequently in peptone medium and grown at 25°C. to provide rapidly growing cultures, but reserve stocks were grown at 16°, at which temperature growth was slow and the medium was not rapidly depleted. In preparation for mating mixtures, these cultures were washed twice by centrifugation with distilled water and concentrated into a small volume of liquid. Infrequently experimental cultures were grown in bacterized lettuce medium, in which mere exhaustion of the bacteria was sufficient to induce the mating condition. Shallow 10-depression slides in petri dishes were used when clones

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were mixed in combinations of two, but deep three-depression slides or small watch glasses were more suitable when groups of clones were mixed together. Test mixtures were generally incubated at 25°C., but temperatures from 16-30° were employed. Continual illumination and total darkness showed no advantages over ordinary day and night laboratory conditions for the incubation of mating mixtures. Test combinations were examined at frequent intervals for conjugating pairs.

A positive reaction between non-selfing clones was interpreted to mean that both conjugants belonged to the same variety. The negative reaction was considered significant only when the known tester mating type and the unknown strain being tested were shown in control mixtures to be in mating condition. Positive results were repeated at least twice, but negative ones several times.

When new strains of unknown mating behavior were collected and ready for mating tests, three separate tests were set up. First, individual strains were distributed into separate slide depressions to determine whether mating occurred within the clone (selfing). Secondly, the unknown strain was mixed separately with any two mating types in the variety or varieties on hand in order to determine to which variety the unknown belonged. And finally, drops from all of the strains in a particular series were combined in one dish, in search of conjugating strains that did not mate with already known varieties. These latter conjugants by definition, constituted a new variety. Each time a new variety was discovered all of the accumulated unknowns were tested against it for reactivity. After the varietal identification was made the mating type was determined by mixing the unknown individually with all types in the variety. If it mated with all types it was assigned a new mating type number, but if the unknown mated with all except one type it was assigned the number of that mating type. Arabic numerals were used to designate varieties and Roman numerals, beginning with I in each variety, were used for mating types. Collections of sexually non-reactive strains were periodically washed and mixed in small groups and *en masse* to afford them the opportunity to mate with other unknowns. Any conjugants were subsequently identified. All sexually non-reactive clones were stained with acetocarmine or acetic-orcein and examined for the presence or absence of micronuclei. Since mating has not been observed in naturally occurring amicronucleate strains, the micronucleus appears to be required for conjugation.

RESULTS

Seven hundred twenty samples of natural water were collected from 46 states and the province of Ontario, Canada. Samples consisted of from one to several jars taken at the same time from the same source. Included in the samples were several from brackish or marine habitats but the majority consisted of a wide array of fresh-water sources distributed in elevation from sea level to 12,800 feet. *T. pyriformis* was found in 154 (21%) of the water samples, from which 3400 clonal cultures were established. The ciliate was found during every month of the year but the highest percentage of positive samples occurred in September (36%) and the lowest in April (9%). Far more samples were collected from Michigan than any other area, and 35% of these were positive for the organism. Unfavorable conditions during postal transit, particularly from distant localities, may have influenced considerably the number of negative samples from the southern and western states. The organism was found about as often in ponds and lakes as in streams, but it was isolated only twice from swamps. The ciliate was not found in marine or brackish samples, or from highly polluted sources, highly alkaline sloughs, or from small temporary puddles.

Strains of *T. pyriformis* from 90 (59%) of the habitats, involving over 2000 clonal cultures, failed to mate in the laboratory under the conditions employed. Nuclear examination made within one to several months after their establishment in the laboratory revealed that 50 (32%) of these were amicronucleate at the time they were inspected. A few collections were lost before the nuclear condition was determined, and 32 habitats yielded micronucleate strains that were never seen to conjugate in any of the trial mixtures made.

Conjugation was observed in strains of *T. pyriformis* from 64 different collections. Two of these series of clones ceased mating before their place in the breeding system could be determined. Eight different collections yielded selfing strains, and the others constituted at least eight non-interbreeding groups, designated as varieties. The number of mating types within a variety varies from two to ten, and a total of 30 new types in seven new varieties were discovered. These are discussed under the separate varietal designations.

Variety 1.—One collection contained *T. pyriformis* that mated with variety 1 stocks. Strains UM221-241, from a lake near Bennington, Vermont, mated with all seven known mating types, except type VI. Since a mating type conjugates with all types except its own type, these strains were shown to belong to mating type VI. Conjugation occurred readily and in large numbers among cultures washed and mixed in the usual manner and incubated at room temperature. Growth in peptone medium has been good, with no depression periods occurring during 21 months of laboratory life.

Variety 2.—Since matings did not occur between

variety 1 types and the unknown strains accumulated, these unknowns were mixed in various combinations. The first pairs found were from a source designated Cape Codder pond, from the vicinity of Woods Hole, Massachusetts. Further tests showed that five clones were of one mating type, designated I, and one clone was of a different type, II. Strains UM3 and UM7 were used as representatives of these, respectively. Another source in the same area, the pond opposite Nobska bathing beach, yielded several strains (UM10-14) of mating type I. Clones from both of these sources had not mated previously and were presumably immature when they were isolated from nature a year earlier. The next two collections of this variety yielded only type I strains. These were isolated from the Mississippi River at Itasca Park, Minnesota, and from a deep roadside ditch in Santa Rosa County, Florida. Two new mating types were found in the group UM350-385, from Gun Lake at Yankee Springs, Michigan. Strain UM350 (type III) mated with types I and II, and strain UM351 (type IV) mated with all three. Thirty-four strains (UM452-487) from Kent Lake at Kenniston, Michigan, yielded mating types I, II, IV, and the new types V, and VI. Strain UM452 was used as type V and UM470 as type VI. In contrast to the Kent Lake situation, Big Turkey Lake at Stroh, Indiana, yielded 29 clones all of mating type III. Mating type VII (UM560) and type VIII (UM557) were discovered from a pond on the golf course at Norman, Oklahoma. They were found with types III and IV, the latter being most numerous. Whitmore Lake, Michigan, yielded the last two mating types discovered in variety 2. Strain UM619 was the only type IX clone collected. Type X (UM616) was first isolated from this lake but was later found in collections from Waterloo Canal, New York, and Grand River near Wellandport, Ontario.

Other Michigan sources from which variety 2 strains were collected were: Muskegon Canal, Saginaw River at Bay City, Diamond Lake at Cassopolis, Winan Lake near Brighton, Grand River at Ionia, and a small pond in the Waterloo Recreation Area. Those from outside the state include a stream at Columbia, Tennessee; an irrigation ditch at Missoula, Montana; Little Duck Creek at Fort Peck, Montana; the reservoir at Logan, Utah; and a pond near White Lake, South Dakota. The 22 collections in this variety came from 11 states and the province of Ontario. Type III was found most often (41%), while types V and IX were rarely found.

Conjugation among strains in this variety usually occurred as readily as among variety 1 strains. At times, however, some mating stocks used as testers failed to conjugate in control mixtures. Non-reactivity often vanished as quickly as it appeared, without its cause being known. No rhythmical recurrence was noticed, but in certain cases there appeared to be a general "weakening" of the strain, which necessitated replacing it with a more vigorous clone of identical mating type. Except for the cases mentioned, growth in peptone medium was good for strains in variety 2.

Variety 3.—Pair formation occurred in a mixture of strains (UM700-718) that had failed to mate with types from varieties 1 and 2. Subsequently four distinct mating types were found in this collection from a small stream in Brookhaven, Mississippi. Strain UM700, mating type I, mated readily and in large numbers with strain UM705, type II, but fewer pairs formed in mixtures with UM701 (III) and UM702 (IV). Conjugation was observed in several other mixtures but very few pairs occurred, and eight strains failed to mate at all until several months later. The degree of sexual activity, or lack of it, found in these strains was probably due to sexual immaturity at the time of their collection. During the first weeks of laboratory life mating was obtained quite readily but with time it became more difficult to obtain conjugation. This may have been due to suboptimal growth in the peptone medium used. The uncertainty of knowing when variety 3 mating types were in mating condition complicated the search for new mating types or strains in this variety. Frequently the mating type of new strains in this variety could not be ascertained immediately because the tester types were not optimally reactive. Growth in bacterized lettuce medium did not alleviate this difficulty, nor was obvious benefit obtained in stock medium through the addition of various growth factors and nutrients required in chemically defined medium(4). An attempt to replace the Brookhaven collection during the summer of 1953 failed, because the small stream in which they had been collected was dry.

Three collections of variety 3 strains were made from Tickfaw, Louisiana; Advance, North Carolina; and Des Moines, Iowa, but their mating types were not determined at the time of their isolation. A new mating type, V, was found in 3 reactive clones from a mountain stream near Brattleboro, Vermont. Strains UM755 and UM756 were of the new type, and UM757 was type I. The other 15 clones isolated at the same time were non-reactive. A second isolation yielded type IV cultures and others of undetermined type. Growth in peptone medium was satisfactory for this latter collection, and conjugation was more predictable than in former collections. Two of four samples from Marquette, Michigan, yielded variety 3 strains. One of these from Dead River (UM791-810) yielded 19 reactive clones. Strain UM795 represented a new mating type, VI, and at a later date one of the undetermined clones was found to be type VIII. Even-

tually five types were found in this collection. Strains UM811-830 from Cox stream, the other Marquette series, yielded types III and VI, and 10 clones of undetermined type.

A reinvestigation of old stocks in this variety revealed that strain UM787, from Townline creek at Leota, Michigan, was a new mating type, VII, and strain UM731, from a stream in Advance, North Carolina, was type VIII. Mating type VIII was also found in a later collection from a fish rearing unit at Auburn, Alabama. A total of 14 collections in this variety were found, but because of difficulties in mating the exact types contained in five of these were not determined. Toward the end of the study it was very difficult to have all eight tester mating types in mating condition at the same time.

Other collections of variety 3 were obtained from Johnson Creek at Raleigh, North Carolina; a stream in the park at Harrisville, Michigan; Rifle River at Omer, Michigan; Jewell Lake at Barton City, Michigan; and Coldwater Lake at Kinderhook, Michigan.

Poor growth and mating behavior of strains in variety 3 may be correlated with the fact, that most of them were collected from running water. Bubbling air through cultures increased the growth rate but did not substantially improve breeding behavior or appearance of animals in these cultures. Changes in media used did not yield lasting effects, but temporary improvements in growth were sometimes seen. Poor-growing cultures frequently contained many ciliates that appeared plasmolyzed. The long immaturity period following conjugation may be due to the slow rate of growth observed. None of the variety 3 exconjugants have mated within 18 months after their isolation. It has been suggested that the irregularities in breeding behavior encountered in variety 3 stocks may be due to intervarietal reactions. A study of the progeny of these matings would be required to resolve that matter.

Variety 4.—Conjugants in a new variety were found in a collection (UM900-927) from Lake Emma at Park Rapids, Minnesota. Strains UM906 and UM914 did not mate with each other but both mated with the other 26 clones tested. Strain UM913 was selected to represent mating type I and strain UM914 for type II. Fifteen strains (UM928-942) isolated at the same time from Little Sand Lake, in the same vicinity, were of mating type I. Thirty-six strains (UM943-978) from Grass River in Canton, New York, were all of mating type II. A new mating type, III, was found in three reactive clones from Island Lake at Brighton, Michigan. Strains UM979-980 belonged to type II while UM981 was mating type III. Sixteen other clones isolated at the same time from this source were sexually non-reactive. A collection from Winan Lake, several miles from Brighton yielded 17 reactive clones (UM986-1014); six were type I, five were type II, and six were type III. Strains UM1015-1034 from White River at Fort Robinson, Nebraska, yielded six strains of type III. The final collection (UM1035-54) in this variety, from a lake near Leonidas, Michigan, yielded 20 clones of type II. Attempts to make another collection of this variety from Island and Winan lakes were unsuccessful. Growth of variety 4 strains in peptone medium was good, but a few strains of type III were observed to grow poorly and react subnormally in mating mixtures.

Variety 5.—In December, 1953, large numbers of conjugants were observed when two old collections were mixed in mating trials. Strains UM24-29 were from a large pond near North Falmouth, Massachusetts. All clones were of the same type, designated I. Clones UM30-41, from a roadside ditch several miles distant, were all of a different type, II. These collections had not been mixed for mating since 1952(3), when they were originally isolated. No sexual activity was noted among these clones at that time. When the routine test for immaturity was attempted, exconjugants could not be isolated because conjugation was lethal between mating types in this variety. All except one of the exconjugants isolated from 30 pairs died. Since the single survivor was found to be mature, it may not have undergone conjugation. Several subsequent attempts to establish exconjugant clones have failed. Further study might show that these strains constitute more than a single variety, but the high percentage of conjugants in mating mixtures and the lack of sexual reactivity with other strains certainly suggest a close relationship among these strains. Growth of both types in peptone medium was very good and conjugation was readily obtained.

Variety 6.—Varieties previously described were discovered by the occurrence of mating between clones from the same source, or, in the case of variety 5, from sources in the same geographic area. This was not the case with the two sources of variety 6 strains. Strains UM1060-1090 were collected from a pond in the Waterloo Recreation Area, Michigan, in May, 1953. They exhibited no sexuality until mixed with clones UM1091-1117 collected almost a year later from a pond east of Mariana, Florida. The 30 clones from Waterloo were of the same type, designated I, and the 27 clones from Mariana were of mating type II. Another collection from Waterloo pond in April, 1954, yielded 23 more clones of mating type I. A third collection was made with the hope of obtaining a different mating type from this pond, but variety 6 strains were not found, only mating type III of variety 2. Since only types I and II were collected in variety 6, other mating types were sought by examining exconjugants from this cross. Sixty exconjugant clones were estab-

lished, of which 27 were type I, 21 were type II, and 9 were a new type, III. Two clones failed to conjugate after 4 weeks of growth at which time all other clones were sexually mature. One clone proved to be a selfing clone. Sub-isolations from this selfing strain yielded other selfing clones. Growth in peptone medium was good for all strains, and mating occurred readily in mature cultures.

Variety 7.—Several days after strains UM1210-1240 were isolated from Buck's Hill branch at Advance, North Carolina, conjugation was observed in the water sample. The 30 clones established contained 4 sexually reactive clones. Strains UM1214 and 1215 were of one type (I) while UM1216 and 1225 were of a different type (II). Tests showed that a new variety was involved. During the first few days following isolation these strains mated readily, but soon sexual activity diminished greatly or failed altogether. This period of sexual inactivity lasted for several months, after which normal mating again occurred and has been maintained to the present. Two attempts to make more collections of the same variety failed. Exconjugant clones established in May, 1954, were still immature in February, 1955. Growth of variety 7 strains in peptone medium resembled that of variety 3 stocks. At times populations reached high numbers and seemed to grow satisfactorily, but often live animals were few in number and many more dead ones sedimented out than usual. Sexual activity appeared to be correlated with good growth, but no method was found to maintain good growth and mating.

Variety 8.—The last new variety found was first detected in a collection (UM1285-1304) from Clear Lake at Jackson, Minnesota. Thirteen of 20 clones proved to be of one type (I), represented by strain UM1285, and 6 were a different mating type (II), represented by strain UM1286. These new types were mixed with all available unclassified strains. Ten clones from Calhoun Lake and 20 from Lake Nokomis, in the vicinity of Minneapolis, Minnesota, were mating type I. These collections had been in the laboratory for almost a year before a complementary type was found. Several type II strains were lost due to contaminations and the remaining strains are not as vigorous as type I stocks. Growth in peptone medium is good for type I stocks but type II strains occasionally grow poorly. Two sets of exconjugants were established for immaturity tests. One set of six clones was grown in aerated bacterized medium that was replaced with fresh medium every 2 days, and the other set of 10 clones was established in peptone medium and transferred only once a month. The bacterized cultures became mature on the 9th day, whereas 10 peptone cultures were immature after 6 months of cultivation. The poor growth of these 10 cultures probably accounts for their failure to attain sexual maturity.

Selfing Strains.—Relatively few collections (5%) of selfing strains of *T. pyriformis* were found. This was surprising in view of earlier reports(10,6). Two selfing strains were found in the 1952 Woods Hole collections which produce viable exconjugants. Two collections of viable selfers were taken from a pond near Pinckney, Michigan. The summer and winter collections of the latter were similar in sexual behavior. Another group of viable selfers was taken from a lake marsh near Whitehall, Michigan. Selfers which failed to yield viable progeny occurred, however, in the collections from Muscatatucket River at Vernon, Indiana, a swamp near Perry, Florida, and River Rouge clones from Plymouth and Detroit, Michigan. Mating in selfing strains did not occur as readily in some clones as in others. Strains from the Pinckney collections lost vigor in the laboratory, but new isolations mated readily. No attempt was made to investigate the selfing collections systematically or to study the behavior of exconjugant progeny.

General Observations.—Two collections of *T. pyriformis* mated when first collected from the wild, but after about 1 week in the laboratory, no more mating occurred. In both cases conjugation had taken place in a composite mixture of clones from a single source. Conjugants in both had been identified to within four reacting strains when mating ceased. After the cessation of mating a nuclear examination revealed that strains from Walnut Creek at Great Bend, Kansas, were amicronucleate, but those from Cashatt stream, Lauada, North Carolina, were micronucleate. It is not known whether the Walnut Creek strains possessed micronuclei when isolated.

Loss of the micronucleus has been observed in one subculture of Strain UM713, a variety 3 tester of type VI. The micronucleus was lost in cultures grown at 20-25°C. for 13 months, but the reserve stock grown at 16° was still normal in this respect. Available evidence suggests that there is a significant relationship between the presence of a micronucleus and the ability to conjugate. This is based on several observations: 1) all conjugating strains have a micronucleus, 2) no amicronucleate strains have been seen to conjugate, 3) loss of the micronucleus in strain UM713 was coincident with loss of sexuality.

An inspection of silverline preparations of representative strains in the eight varieties reveal only infraspecific variations (Corliss, personal communication). Representative strains from varieties 3 and 7 exhibit a lower mean number of ciliary rows than the others, and the 2°PM's (second part of Meridian) were very definite and heavy. Strains in the other 6 varieties were alike in silverline structures, with 19-23 ciliary rows and the 2°PM's rare or absent. Variety 5 strains were

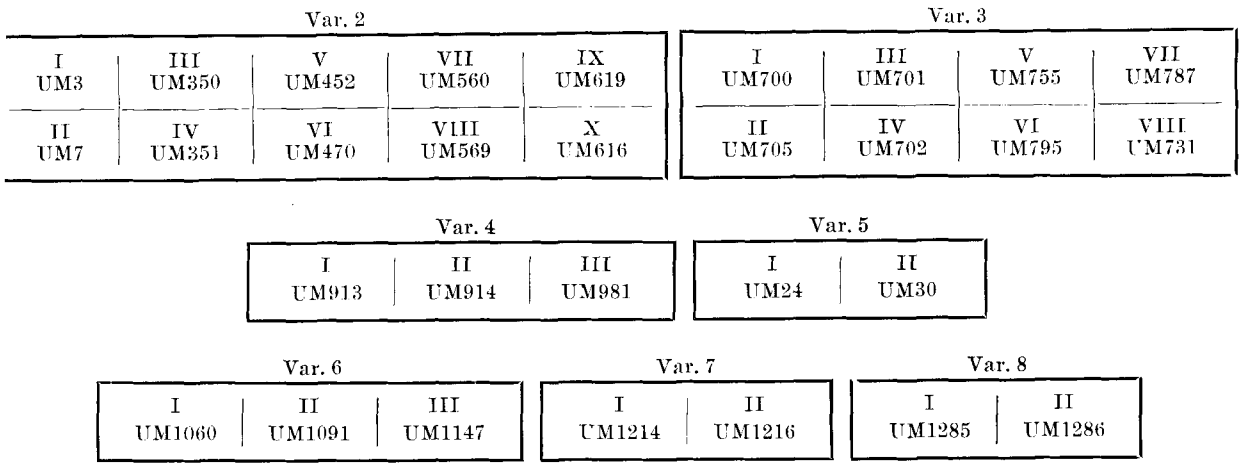

Fig. 1. A summary of new varieties and their mating types in *T. pyriformis*. Strains listed are those first found exhibiting the particular mating behavior.

considerably smaller than the mean size, and varieties 3 and 7 strains were somewhat larger than the mean. Partially double (doublets) animals and other anomalies were far more common in the latter varieties. Clones isolated directly into bacterized medium, without having been exposed to antibiotics, were of the same general appearance as those grown in peptone medium. In mixtures containing abnormally shaped individuals the smaller and more nearly normal ones were usually the only ones that mated. Various combinations of doublets and singles were seen; triplets were common, and conjugating groups of 4, 5, 6, and 7 were seen. Attachment was always near the anterior end of the body. Autogamy was not observed in any strains investigated, nor was mass clumping of complementary types seen in any varieties of *T. pyriformis*.

A summary of the new varieties and their mating types is shown in Fig. 1. Strains listed are those first found exhibiting the particular mating behavior.

DISCUSSION

A. *The breeding system in T. pyriformis.* The breeding behavior in *T. pyriformis* exhibits many similarities to that found in other ciliates. The nature of the mating type resembles that described in *Paramecium aurelia*(15) and *P. bursaria*(11). The mating type of sexual strains collected has remained constant. Conjugation occurred when two complementary types were mixed under appropriate conditions, but was not induced by the action of fluid from one culture upon another of a different type as has been reported(16) in other cases. The mode of mating type inheritance(13) resembles that in *Paramecium* but studies have not been conducted to determine whether there are different modes of inheritance as in Groups A and B in *P. aurelia*(16). A further similarity to *Paramecium* and *Euplotes*(16) is the occurrence of groups (varieties) of interbreeding mating types in *T. pyriformis*. No instances of intervarietal matings have been observed but the lethality of matings in variety 5 might suggest intervarietal crossing such as those reported in various species of *Paramecium*(7,16). Lethality of conjugation was observed in certain ones of the selfing strains collected but viable progeny resulted from matings in the other eight varieties thus far reported(3,5, 9). An examination of the chromosome complement of variety 5 strains(14) revealed that these chromosomes were similar in size, shape, and number to those of 3 other varieties of *T. pyriformis* examined.

No evidence for degrees of compatibility within a variety was obtained, with the possible exception of variety 3. It is believed that the poor sexual reactions obtained in this variety resulted from the poor physiological condition of these stocks. Types I, II, and VIII were usually reactive but others frequently failed to conjugate. A progressive decrease in sexual vigor with laboratory life was sometimes apparent when newly collected clones were compared to older mating stocks. This was observed in selfing strains, as well as in others. Since this weakening sometimes occurred shortly after the clone was established in the laboratory, it was probably due to unfavorable conditions, rather than senescence. In spite of difficulties encountered, all breeding stocks except type III of variety 6, are those isolated from nature, and were not the result of sexual reproduction in the laboratory. On occasions it was necessary to replace certain testers with similar stocks exhibiting more vigor. It may well be that in time it will be necessary to replace breeding stocks with appropriate vigorous exconjugants. How-

ever, all strains do not appear to age at similar rates. Variety 1 strains WH6 and WH14(4) have retained sexual vigor during their 3 years of laboratory life without the intervention of sexual reproduction.

The 30 new mating types in seven varieties reported in this paper,[1] in addition to seven types in variety 1 (4, 13), type XI in variety 2, and 5 types in variety 9 (5), bring to 43 the types known in 9 varieties of *T. pyriformis*. This exceeds those reported for *P. aurelia* (1) or *P. caudatum*(8) even though these latter species exhibit as many or more varieties. In *T. pyriformis* the number of mating types found in each variety does not conform to the 2^n number per variety reported in the multiple breeding system of *P. bursaria*(12). Probably a more extensive search would yield added types, especially in varieties 1, 2, 4, 6, and 9 if this phenomenon obtains in all ciliates exhibiting a multiple breeding system and caryonidal inheritance.

B. *Distribution.* *T. pyriformis* was collected from 35 states and from Ontario, Canada. The 21% of samples positive for the ciliate compares well with results obtained in Mexico, Panama, and Colombia(5). A higher percent (35%) of positives was found in Michigan, perhaps because of better care given the field samples. In a collecting program of the sort used, many individuals make collections from the wild, and results obtained depend to a large measure on the choice of microhabitat for sampling and the care given the sample collected. Samples taken repeatedly from certain ponds or streams suggest that the ciliate is absent from some places but present in others in the same area, as was found in areas of Panama(5). Prolonged hot weather and drought conditions in parts of the collection areas in the south and west apparently reduce natural populations of *T. pyriformis*. Very few or none were collected under these conditions.

It is probably true that the different varieties in the species are more widespread than the present data indicate. Intensive search in a given geographic locality would likely yield several varieties of *T. pyriformis*. Varieties 1, 2, 5, and selfers were collected in the vicinity of Woods Hole, Massachusetts; varieties 1, 2, 4, and 8 in Minnesota; and varieties 2, 3, 4, 6 and selfers in Michigan. Yet, no field sample yielded more than one variety. Elliott and Hayes(5) have, however, obtained varieties 2 and 9 in one sample from Panama.

Some evidence suggests that more than one variety occurs concurrently in a given pond or lake. Two local examples illustrate this point. First, Winan Lake originally yielded only variety 4 strains, but several months later only variety 2 strains were obtained from the same collection site. Secondly, the pond at Waterloo Recreation Area yielded variety 6 strains on two different occasions, then in the third collection variety 2 strains were found. A similar situation occurred with reference to amicronucleate and micronucleate strains. Two collections several months apart from Whitmore Lake, Michigan, yielded amicronucleate strains. The third sample, 6 months after the previous one, yielded a good representation of variety 2 mating types. The reverse was the case from Little Sand Lake, Park Rapids, Minnesota. Variety 4 strains were first isolated, but on a subsequent collection only amicronucleate strains resulted. It appears that these varieties were occupying different microhabitats if they occurred concurrently, due possibly to intervarietal competition or to the existing environmental conditions. On the other hand, sometimes as many as five or six diverse mating types in a given variety were discovered in the same field sample. Samples taken from Waterloo pond were deliberately diverse and numerous in search of more than type I of variety 6, but no other mating type in this variety was collected from this pond.

Variety 2 strains were most widely distributed geographically and in diversity of aquatic habitats. These were collected from Montana and Utah eastward and from Canada to Florida. Elliott and Hayes(5) identified only this variety from Mexico, and varieties 2 and 9 (a new variety) from Panama and Colombia. In the current study, variety 2 was found in 10 streams and 12 standing sources. In contrast, variety 3 was collected from 12 streams and only two lakes (in Michigan). The fact that variety 3 strains occurred more frequently in running waters may be associated with certain specific physiological demands. This could account for poor growth in laboratory cultures of varieties 3 and 7 strains. It bears mention that varieties 1, 5, 6, and 8 were collected only from ponds or lakes; and that variety 4 was taken five of seven times from lakes. Cultures from these varieties grew and reacted well in the laboratory. Collections in varieties 1, 4, 5, and 8 were limited to northern states, but the limited number of these collections precludes comment on the significance of this observation. Similarly restricted sources for certain varieties of *Paramecium* have been reported(1,8). The degree to which *T. pyriformis* is distributed over the surface of the earth is unknown, but it seems to compare favorably with other ciliates widely collected. Its small size and sparse population in water samples probably accounts for its frequent omission from faunal check lists.[2]

[1] A portion of these findings appeared in abstract form(9).

[2] The writer is sincerely appreciative of the many field collections sent by numerous persons during the course of this investigation. Thanks are extended to Drs. A. M. Elliott, D. L. Nanney, and R. E. Hayes for helpful suggestions during the pursuance of the problem and preparation of the manuscript.

REFERENCES

1. Beale, G. H. (1954). The genetics of *Paramecium aurelia*. Cambridge University Press, Cambridge.
2. Corliss, J. O. (1953). Silver impregnation of ciliated protozoa by the Chatton-Lwoff technic. *Stain Technol.*, **28**, 97-100.
3. Elliott, A. M. & Gruchy, D. F. (1952). The occurrence of mating types in *Tetrahymena*. *Biol. Bull.*, **103**, 301.
4. Elliott, A. M. & Hayes, R. E. (1953). Mating types in *Tetrahymena*. *Biol. Bull.*, **105**, 269-284.
5. ——— (1955). *Tetrahymena* from Mexico, Panama, and Colombia, with special reference to sexuality. *J. Protozool.*, **2**, 75-80.
6. Elliott, A. M. & Nanney, D. L. (1952). Conjugation in *Tetrahymena*. *Science*, **116**, 33-34.
7. Gilman, L. C. (1949). Intervarietal mating reactions in *Paramecium caudatum*. *Biol. Bull.*, **97**, 239.
8. ——— (1954). Occurrence and distribution of mating type varieties in *Paramecium caudatum*. *J. Protozool.*, **1**, (Suppl.), 6.
9. Gruchy, D. F. (1954). The system of breeding relations in *Tetrahymena pyriformis*. *J. Protozool.*, **1**, (Suppl.), 2.
10. Horn G. F. (1951). Conjugation in *Tetrahymena geleii* S. Master's Thesis, University of Washington.
11. Jennings, H. S. (1938). Sex reaction types and their interrelations in *Paramecium bursaria*. I. *Proc. Natl. Acad. Sci.*, **24**, 112-117.
12. ——— (1939). Genetics of *Paramecium bursaria*. I. Mating types and groups, their interrelations and distribution; mating behavior and self sterility. *Genetics*, **24**, 202-233.
13. Nanney, D. L. & Caughey, P. A. (1953). Mating type determination in *Tetrahymena pyriformis*. *Proc. Natl. Acad. Sci.*, **39**, 1057-1063.
14. Ray, C., Jr. & Elliott, A. M. (1954). Chromosome number of four varieties of *Tetrahymena*. *Anat. Record*, **120**, 228.
15. Sonneborn, T. M. (1937). Sex, sex inheritance and sex determination in *Paramecium aurelia*. *Proc. Natl. Acad. Sci.*, **23**, 378-385.
16. ——— (1947). Recent advances in the genetics of *Paramecium* and *Euplotes*. *Advances Genet.*, **1**, 264-358.

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Gregarines Found in the Honey Bee *Apis mellifera* Linnaeus in Venezuela.

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SUMMARY. Gregarines were found for the first time in the honey bee *Apis mellifera* L. in Venezuela. The parasites attacked the inner wall of the ventriculus of the adult bees, causing heavy losses in apiculture in October 1954 and June 1955. The disease produced was called gregarina disease of the honey bee (in Spanish "gregarinosis de la abeja").

IN OCTOBER 1954 and June 1955, great quantities of sick and dead honey bees were observed in apiaries in Venezuela near Maracay (Longitude W.67°, Latitude N. 10°, altitude 450 m.). The author found about 100 sick bees daily in October 1954 in front of 4 hives in an apiary where he observed the bees closely. Microscopical investigations showed that the trouble was caused by gregarines parasitizing the ventriculi of the bees.

MATERIALS AND METHODS

The sick, dead and healthy worker bees were collected daily in the study apiary and also occasionally in neighboring apiaries within a distance of 15 km.

The bees were first examined microscopically for suspected *Nosema apis* Zander or *Malpighamoeba mellificae* Prell, which had been found previously, then for the generally known diseases caused by fungi, bacilli or viruses as well as for poisons, but all of the examinations were negative.

Upon methodical examination of all parts of the body, gregarines were found in the smears from the first segments of the ventriculus, near the oesophagus. They were also found within the ventricular wall as well as in the hemocoel of the same region and also on the Malpighian tubules. In order to determine the correct location of the gregarines, longitudinal sections were made of the entire body of the bee.

After the identification of gregarines in the digestive tract of the honey bees, all species of insects associated with the honey bees in the hives were investigated in order to find the reservoir hosts of the gregarines. The microlepidoptera *Galleria mellonella* L., *Achroia grisella* Fabr., *Plodia interpunctella* Hbn. and many species of Blattidae (cockroaches) *Periplaneta americana* L., *Periplaneta australasiae* Fabr., *Panchlora nivea* L., *Blaberus giganteus* L., *Blaberus discoidalis* Serville, *Blattella germanica* L. and some other unknown Blattidae collected in the hives were examined especially. Among these, only the Blattidae are known as hosts of gregarines(1-7).

The wet unfixed smears with gregarines were examined either without stain or else they were stained with fast green or erythrosin. Tissues from parasitized honey bees were fixed in Bouin's, embedded in paraffin blocks, sectioned at 10 microns and stained with Mallory's haematoxylin. Tissues examined microscopically were the head, thorax and all parts of the abdomen. Photographs were made of typical preparations.