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The Distribution of *Tetrahymena pyriformis*[1]

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SYNOPSIS. This paper is a brief account of both amicronucleate and sexually active strains of *Tetrahymena pyriformis* and their distribution with some comments on their possible evolution.

PROBABLY most early microscopists who studied fresh water protozoa saw *Tetrahymena pyriformis*. From the time André Lwoff grew this ciliate in axenic culture in 1923 (21) many thousands of strains have been isolated from fresh water in various parts of the world, indicating its ubiquitous occurrence. Like *Paramecium* and other ciliates which have been carefully examined, this species includes many reproductively isolated populations, each with a sharply defined gene pool. If the concept of a biological species is adhered to, each would qualify as a species. The relative merits of assigning species names to the many strains of such interbreeding populations of protozoa was discussed in detail by Sonneborn (33) and Hairston (17). For a long time they have been referred to as *varieties* but currently the term *syngen* is being used and probably is more appropriate. For that reason these populations of *T. pyriformis* will be referred to as syngens.

The so-called "classical" amicronucleate strains, which have been used so extensively as experimental tools, were all isolated from natural waters in the United States* with the exception of 4, 3 of which were found in France and one in England (5). Since these strains do not conjugate they tell us little about their relationships. The discovery of sexuality provided organisms with the potential for examining the genetic constitution of different populations (syngens) taken from various regions of the earth. This information could give us some understanding of the relationships among the populations as well as some insight into their evolution.

The 1st interbreeding population of *T. pyriformis*, taken from fresh water samples collected on Cape Cod, Massachusetts, was discovered in 1951 (11). Since that time nearly 1,300 fresh water habitats from the major continents, as well as Pacific Islands, have been examined for this ciliate. The area covered can only be roughly approximated at best but probably was something less than 4,000 square miles. This means that water samples taken from small streams, rivers, ponds, lakes, etc., may be representative of the surrounding area. Thirty-nine percent of the habitats contained the ciliate, but this varied widely (10-70%) in different localities. Among the 7,115 clones isolated, 50 mating types were categorized to 12 syngens. Some minor structural and physiological variations exist among them. Whereas an attempt will be made to distinguish them from one another based on the few characteristics that have been revealed, the one single character that clearly separates them genetically is their breeding patterns. There is little, if any, gene flow among the syngens because cross-breeding rarely occurs.

All syngens have certain characteristics in common. *T. pyriformis* feeds on bacteria in nature but it flourishes axenically in test tubes when offered different bacteriological media. It can also be maintained on a chemically defined medium consisting of 20 nutrilites (9, 10). Nearly all strains have these nutritional requirements, hence have been designated the "wild type." A very small number have slight variations from this pattern and have been called "mutants" (9, 10). Such mutants are not correlated with syngens; consequently nutritional requirements cannot be used as a syngenal character.

Structurally all syngens are very similar. They are usually piriform, with variations possibly sufficient in some cases to be used as a syngenal character. In a study of their dimensions under controlled conditions (unpublished) it was found that, whereas there is a great deal of overlap, some syngens can be identified by size. A specified number of ciliates (100/ml) were inoculated into a 2% proteose-peptone medium and incubated at 25 C. When they were in logarithmic growth (48 hrs) representative samples (100 cells) were measured (length & width). It turned out that some syngens were longer (syngens 6, 8, 9) than others (syngen 10) but most fell between the extremes. The ciliary meridians vary from 17 to 23, but this amount of variation may occur within one clone, and hence is of no value as a diagnostic character. The buccal apparatus is similar in all varieties (5, 6). The irregularly spherical macronucleus and the smaller micronucleus lying in an indentation in the macronucleus, are very much alike in all of the syngens. The larger ciliates have a somewhat larger macronucleus than the smaller ones, whereas the micronucleus seems to be the same size in all. These are casual observations without statistical data.

The time required for conjugation to begin, once the mating types are mixed, is called the refractory period (12). The refractory period is relatively consistent for all mating types in a syngen when clones are grown axenically in broth and washed twice in distilled water before mixing (unpublished). This character can be used to distinguish some syngens. It is longest in syngens 8 and 9 (36-46 hrs.) and shortest in 1 and 12 (2-4 hrs.).

The length of time of sexual inactivity following suc-

[1] The information reported in this paper includes both published and unpublished data that resulted from support by research grants (E-1416) from the National Institute in Allergy and Infectious Diseases, National Institutes of Health, the United States Public Health Service. Some of the contents will appear in Chapter 12. *The Biology of Tetrahymena* (ed., A. M. Elliott) (New York, Appleton Century Crofts, 1970)

* As a result of the encouragement given by Professor R. P. Hall, the author isolated one of these strains (E) from a pond in Van Cortland Park in New York City in 1931.

cessful conjugation is defined as the immaturity period (33). This sometimes differs among syngens and can be used as a distinguishing character in some syngens, altho many require approximately the same period of time. In some syngens no obvious immaturity period has been found. Exconjugants, when permitted to undergo vegetative fission until a sufficient number are available for a test, will mate immediately. Others require varying periods of time before they are sexually mature, some as long as 30 days when frequently subcultured. Those with no immaturity period are thought to be inbreeders because they have little or no opportunity to disperse before they may mate again (33). On the other hand, those with a long immaturity period may spread into new environments before conjugation can again occur, and thus are thought to have outbreeding tendencies. These breeding characteristics may influence the distribution of many of the syngens.

The immaturity period as a syngenal character is not completely reliable owing to certain cytogenetic errors that frequently occur. Sometimes the cells mate but fertilization does not occur because of defective micronuclei, faulty meiosis, or failure to exchange pronuclei. This phenomenon is called genomic exclusion by Allen et al. (1, 2). When strains suffering from this aberration separate following mating, the exconjugants have no immaturity period. This could account for some of those syngens that seem to have no immaturity period. Such errors in conjugation must be considered when using the immaturity period as a syngenal character. At the time the data were collected on the syngens described below this phenomenon was unknown, hence some adjustment may be warranted.

The system of mating type inheritance has been studied in some syngens (27). Since the data are incomplete for all of the syngens except syngen 1, it is inadvisable to include the available information as a syngenal character.

Tolerance to temperature can be used to distinguish some syngens as pointed out by Holz et al. (18). They studied the high temperature tolerance of all of the mating types of syngens 1 thru 9. Their work was verified and extended in our laboratory (unpublished). All mating types in the 12 syngens were subjected to low (2-6 C) and high (37 C) temperatures for 10 days. They were checked periodically for survival and growth. All mating types grow well at 25 C. Some will tolerate and even grow at 2 C and have been called "cold strains." Others, identified as "hot strains," survive at temperatures as high as 40 C. Low temperature tolerance may be significant in the distribution of some syngens, as discussed later.

The geographic distribution of some syngens is sufficiently precise to be used for identification. This is particularly true of syngens 9, 10, 11 and 12. Syngens 1, 2, 7, and 8 are confined to the American continents, while 3, 4, and 6 are distributed over 4 continents. In other words, one would expect to find syngen 10 only in England, syngens 11 and 12 only in Australia, syngens 1, 2, 7 and 8 only in the Americas, but 3, 4 and 6 may be found in Eurasia and Africa as well as in the Americas.

The characters described above are the only ones that have come to our attention that are useful in distinguishing one syngen from another.

Syngen 1. Sexually active strains of *T. pyriformis* were 1st isolated from fresh water ponds near Woods Hole, Massachusetts in 1951 (11). They consisted of 3 strains (WH_6, WH_{14}, WH_{52}) which were categorized as mating types I, II, and III, respectively, and the interbreeding group was assigned to syngen 1. Shortly thereafter 2 other strains, PR_1 and PR_2, were found in a lake in northern Minnesota. These belonged to mating types I and II. Seven mating types were subsequently derived in the laboratory from WH_6 and WH_{14} by Nanney and Caughey (27). Gruchy (16) reported mating type IV (strains UM221 and UM241) from a Vermont habitat. Nanney (personal communication) found ALP-4 in Michigan and IL-12 in Illinois. More recently, Phillips (50) reported 6 strains from Illinois. The distribution of this syngen seems to be sparse tho widespread in the United States.

As this syngen has been investigated more than any other, a great deal is known about its breeding system as well as its genetics. It has a short refractory period (2-4 hrs.) and has an immaturity period lasting thru at least 80 fissions (26). The maturity period lasts for several years as indicated by the fact that WH_6 and WH_{14} still conjugated after 17 years in culture, altho offspring viability dropped from 17% to less than one percent (unpublished).

None of the strains tolerate temperatures of 6 C but all grow at 37 C and some survive at 40 C providing the cells are exposed to 35 C before being placed at the higher temperature (27).

Syngen 2. Representatives of this syngen have been found in widely separated localities in the Americas; it appears more often in collections than any other syngen. It was 1st found in water samples taken from Cape Cod, Massachusetts and later from Minnesota, Michigan, Indiana, New York, Montana, Utah, South Dakota, Oklahoma, Florida and Ontario, Canada (16). It has also been found in Mexico and Central America (13). It has not been taken from habitats outside the Americas.

Ten mating types were originally identified by Gruchy (16) and an 11th by Elliott and Hayes (13). However, when these were reexamined by Hurst (19), only 9 were considered valid, V and XI being relegated to obsolescence. Even so, it contains the largest number of mating types of any of the syngens, emphasizing its outbreeding potentialities. It has a variable refractory period (4-12 hrs) and an immaturity period that is also highly variable. Hurst (19) found that some strains became mature after 12 divisions whereas others required as many as 192 fissions before they would mate again. This extreme variation in the immaturity period may have significance in explaining the widespread distribution of this syngen in the Americas. This will be discussed later.

There is some variation among the strains regarding their tolerance to temperature. Some will grow poorly at 6 C, while others survive 2 C. None can withstand 37 C,

but Holz et al. (18) reported that all mating types survive and grow well at 35 C.

Syngen 3. Members of this syngen were isolated from habitats in Mississippi, Louisiana, North Carolina, Alabama, Vermont and Michigan (16). Later it was found in Austria (14). In all, 8 mating types were identified by Gruchy (16). However, an intensive study of the breeding system by Byrd (3) revealed only 7, and he was unable to add any new mating types. The refractory period is relatively short (4-6 hrs). This syngen has strong outbreeding tendencies documented by its long immaturity period (158 fissions for one-half to become mature). It remains mature and sexually active for a long time (at least 13 years). Moreover, its progeny survival following conjugation is enhanced more when outbred than inbred. These facts, together with its numerous mating types and wide distribution, emphasize its outbreeding economy.

A few strains tolerate low temperatures, surviving at 6 C. Holz et al. (18) reported that all mating types grow moderately well at 35 C. In our laboratory none survived at 37 C.

Syngen 4. Strains belonging to this syngen were taken from Minnesota, New York, Nebraska and Michigan (16). It was later found in England (8) and more recently in India, hence it also has a wide distribution. Three mating types were isolated in 1954 (16), all of which showed symptoms of senility after several years of culture. A rigorous breeding program has resulted in reactive mating types which yield viable progeny. In 1954 Professor B. R. Seshashar very generously sent clones of *T. pyriformis* taken near Delhi, India. These all turned out to belong to syngen 4, mating type II; they are the only sexually active strains taken from Asia.

Members of this syngen have a short refractory period (4-6 hrs.) and an immaturity period of approximately 3 days of laboratory culture which means that it may have none at all (but see Allen et al. 2) It is possible that these strains are ridden with defective micronuclei which would mean that fruitful conjugation did not occur in our tests which would explain the lack of an immaturity period. Its long maturity period (13 years) and wide distribution suggests that it may outbreed in nature.

Members of this syngen grow well at 6 C and survive at 2 C but none tolerates 37 C. Mating type III, tho not I and II, survives 35 C (27).

Syngen 5. When 1st isolated from natural habitats in Massachusetts, mating types I and II failed to produce viable offspring (16). Numerous breeding experiments since then have also been negative. It has never appeared in other collections, and hence may not be a true syngen. This may be a rare case of inter-syngenal mating.

Syngen 6. Members of this syngen have a rather interesting history. Mating type I was isolated from Michigan waters and II from a Florida habitat (16). A subsequent effort was made to derive additional mating types from these 2 which was successful, yielding mating type III. Other strains belonging to mating types I and II were found in England, France, Holland and Italy (8). More recently (1964), thru the courtesy of Dr. R. E. Kuntz, water samples taken within 10 miles of Cairo, Egypt contained *T. pyriformis.* These all belong to syngen 6, mating type II (unpublished). This syngen, like syngens 3 and 4, is widely distributed over a number of continents.

The refractory period is relatively short (4-6 hrs) and the immaturity period lasts from 4 days (31) to 4 weeks (16, 34) in routine culture. The maturity period is apparently very long because our original strains still mate after 15 years in culture. However, they have steadily declined in sexual activity over the years. Efforts to revitalize the various strains of this syngen by an intensive breeding program were successful in 1964. What its status is at present is not known.

All strains tested grow well at 6 C and some survive at 2 C. None survive at 37 C.

Syngen 7. Representatives of this syngen were all isolated from a single habitat in North Carolina (16). Two mating types were established. Outka (30), in a careful analysis of the breeding system, derived mating types III and IV from the original parental types. He also found mating type instability, which is characteristic of this syngen. Physiologic conditioning by adjusting the osmolarity with sucrose was required for successful conjugation. The refractory period is unusually long (24 hrs) and no immaturity period was observed. The apparent instability of strains in this syngen and the lack of an immaturity period may be accounted for by faulty micronuclei in the parental strain as discussed by Allen et al. (2).

Our present 3 mating types are able to barely survive at 6 C, but die at 37 C.

Syngen 8. Strains belonging to this syngen were isolated from several habitats in Minnesota, from which 2 mating types were established (16). Later another mating type, III, was added by Orias from Michigan (28, 29). He also found members of this syngen in Wyoming but did not report the mating type. The refractory period was shown by Gruchy (16) to be 4-6 hours at 25 C but Orias said that it is very long (46 hrs). He also found that the immaturity period lasts for 120-150 fissions in routine culture. It probably outbreeds in nature.

Strains of this syngen tolerate 2 C and will grow at 6 C. They cannot survive at 37 C.

Syngen 9. Five mating types belonging to this syngen were 1st identified from 19 clones isolated from habitats in Panama and the Canal Zone in 1954 (13). Among a number of clones taken near Bogota, Colombia 4 belonged to mating type IV. More recently, mating type III was found on Oahu Island, Hawaii and Viti Levu, Fiji (15). Since the syngen has been found only near or within the tropics, it may be restricted to these latitudes.

It possesses one structural character which separates it from all others investigated. During the later stages of conjugation the degenerating macronuclei lie in the anterior region of each conjugant, whereas in all other syngens these structures are located in the posterior region.

Members of this syngen have a long refractory period

(36 hrs) and no immaturity period could be found (14). Since progeny survival is about the same whether they are inbred or outbred they seem to have both inbreeding and outbreeding characteristics.

Strains of this syngen do not survive at 6 C nor will they tolerate high temperatures (37 C).

Syngen 10. Water samples taken from the tributaries of the Thames River in the vicinity of London, England, contained members of this syngen (8). From the small number of habitats examined, which contained 4 reactive clones, 2 mating types were isolated. It was not found in any other collections on the Continent of Europe, and hence may be confined to the British Isles. Syngens 4 (mating type III) and 6 (mating type II) were collected in the same localities in England as well as on the Continent. It is interesting that these 2 syngens have successfully spread to England but syngen 10 apparently did not succeed in reaching the Continent.

Members of this syngen are generally smaller than representatives of other syngens. Their mean length is 36 μ, which is the lower limit for all syngens. There is a slight difference in the growth rate between the mating types, I being slower than II. The refractory period is 4-5 hours at 25 C and no immaturity period could be found. When 1st isolated in 1958, mating was vigorous, but at that time no effort was made to determine progeny survival. However, in 1963 mating could be induced in only one cross of the several strains in our collection and none of the progeny survived. Apparently, aging had progressed rapidly during the 6 years of laboratory culture. With only 2 mating types, no immaturity period and a very limited distribution, one may conclude that syngen 10 is an inbreeder.

The strains grow poorly at 6 C and die at 37 C.

Syngen 11. A rather extensively collecting schedule in Eastern Australia in 1960 revealed that this syngen is rather generally distributed in New South Wales (15). It was not found in Queensland or in the Northern Territory (near Darwin). It may be confined to the southern latitudes because the sampling was sufficiently extensive to reveal its presence if it occurred in any abundance in the northern provinces. This syngen, as well as syngen 12, has been found only on the continent of Australia. This could be expected owing to the long geographic isolation of this land mass.

Three mating types were established from the many clones isolated. The refractory period is short (4-6 hrs) and no immaturity period could be found. The ciliary rows are somewhat fewer (16-19) than in other syngens (17-23) but this may not be a reliable character since there is considerable variation even within a strain (15). Conjugation is strikingly out of synchrony and the conjugants remain attached unusually long (24-48 hrs). No information was obtained on progeny survival when members of this syngen were 1st isolated. However, 4 years later approximately 5% of the offspring survived (unpublished). The strict limitation of this syngen to Australia is adequate for identification purposes.

All of the strains grow at 6 C and some survive at 2 C but all die at 37 C (unpublished).

Syngen 12. Representatives of this syngen were also discovered in Australia, being taken in both New South Wales and Queensland (15). They seem to be more widely distributed than syngen 11, altho the actual numbers isolated were fewer (38%) than syngen 11 (62%). Like the latter syngen, the number of ciliary rows is somewhat less than that of other syngens. Conjugation is sharply synchronized, the refractory period is short (2-4 hrs) and no immaturity could be found. The conjugants separate within 24 hours. These characters alone separate syngens 11 and 12.

Strains cannot survive at 6 or 37 C, and hence are the least tolerant to temperature variation of all of the syngens.

Unidentified *T. pyriformis. T. pyriformis* clones were isolated from all land masses where collections were made but a great many of these (30-50%) turned out to be amicronucleate and were discarded. Even tho numerous clones were micronucleate it was not possible to assign all of them to syngens owing to their failure to mate either among themselves or with the known testers. This was probably due to the fact that they belonged to a new syngen and only one mating type was isolated or they were senile when isolated, or for reasons that are not clear. Non-reactive micronucleate clones were taken from habitats in Kwangtang Province, Hong Kong, Philippine Islands near Manila, New Zealand (both North and South Islands), Japan, Switzerland, Sweden and Central Africa. Dr. Robert Kuntz very kindly supplied water samples from Africa, in addition to those from Egypt mentioned earlier, which contained micronucleate *T. pyriformis.* Clones isolated from habitats in Tanganyika unfortunately did not mate with our 12 syngenal testers nor among themselves. Those isolated from water samples taken in Nairobi, however, did mate among themselves but not with our testers. They may well be a new syngen.

DISTRIBUTION

From the foregoing account it seems clear that *T. pyriformis* is ubiquitous in its distribution. It occurs in fresh water ponds, small streams, lakes, and rapidly moving water. It may live as a facultative parasite. It has been found in natural waters at temperatures ranging from 4 C (Kiruna, Sweden) to 30 C (Panama), and in the laboratory the hot strains thrive at 37 C. It exists at sea level and at 10,000 feet in mountainous streams. It is probably safe to assume that it has invaded nearly all fresh water habitats. How it, as well as other species of protozoa, has been able to spread over the surface of the earth is a fascinating problem about which we know next to nothing.

Based on structure it may be assumed that all of the syngens are closely related and that they probably had a common origin. If this is so, and most taxonomists would agree, then geographic isolation thru time must have been the primary mechanism in bringing about the numerous syngens we see today. *T. pyriformis,* being a highly prolific organism with strong powers of movement, has the capacity to become widespread. The constant shifts in

the earth's surface with concommitant alterations in patterns of flow of water thru geologic time have also facilitated its ubiquitous distribution over the present land masses. It is not so easy to explain how it reached isolated land masses such as the Pacific Islands.

Any attempt to trace the evolution of the numerous syngens from a common ancestor, if one exists today, is fraught with insurmountable difficulties owing primarily to a lack of sufficient data both on syngenal characteristics and on their present location. As was pointed out earlier, we do not know enough about the genetics of each syngen and the collections have been too sparse in many regions of the world to be representative altho they may be in some, the United States for example. Even with the current fragmentary data it is possible to show some relationship between the known syngens.

A. *The Americas*: It was pointed out earlier that syngen 2 has the widest distribution of all in the American continents. It has been found in collections from Massachusetts to Utah and from Canada to Panama. All strains in this syngen interbreed freely, suggesting that dispersal of mating types has been relatively recent. No members of the syngen have been found elsewhere, hence it seems to be confined to the Americas.

Syngens 1, 7 and 8 have been found only in the United States and they seem to exist as isolated populations. Syngen 1 is the most widespread. It has appeared in collections from Massachusetts, Vermont, Minnesota, Michigan and Illinois. Syngen 8 has been taken only in the midwest, Michigan, Minnesota and Wyoming. Syngen 7 seems to be the most isolated one of all, having been found once in a single habitat in North Carolina.

Syngens 3, 4 and 6 are widely distributed over the United States (16) as well as in other parts of the world (see below). These 3 syngens tolerate low temperatures. This character may have made it possible for them to migrate to other continents as is discussed later.

There are certain incompatibilities among strains of syngen 6 that may have some bearing on its distribution. The European syngen 6 strains mate readily among themselves but only 6/II strains mate with American 6/I strains (8). No other crosses between the American and European strains were observed. The African 6/II strains cross readily with both the European and American 6/I strains. In all these crosses the progeny, if any, failed to survive the next inbred cross, indicating genic incompatibility. However, vigorous mating with 1st generation survival dictates close affinities and it is likely that all the strains studied belong to syngen 6. This suggests that the American, European and African strains originally belonged to the same gene pool. With time lethal genes have accumulated in sufficient numbers to prevent fruitful conjugation at the present time. This may be interpreted to mean that at one time syngen 6 mating types were distributed thruout Eurasia, Africa and North America as a part of a single gene pool but today, as a result of geologic isolation, it is showing genetic separation into 2 syngens.

Syngen 9 was found only in Central and South America and on 2 Pacific Islands (see below). It has not appeared in collections taken from northern latitudes, hence it may be considered to be confined to the tropics.

B. *Eurasia and Africa*: Of all the syngens that occur in the Americas today, only 3, 4 and 6 have been found in Eurasia and Africa. The collections from Asia and Africa were very few but in Europe they may be sufficiently numerous to include the commonly occurring syngens (8). An interesting question is, how did these 3 syngens reach such distant lands when the other American syngens did not? Some speculation to a possible answer to this question may be warranted even tho the data are fragmentary. Eurasia and North America were continuous via Bering Strait thruout most of the Tertiary Period and there was much mammalian migration during the Eocene (23). This land connection existed again during the Pliocene and Pleistocene (32). It is reasonable to assume that syngens 3, 4 and 6 could have arrived in Eurasia over this land bridge, paralleling the path taken by the horse and other animals. Subsequently, syngen 6 could have reached Africa following its spread thruout Asia. Syngens 3 and 4 may have reached this southern continent also but they were not found in the very limited collections made. Since all of the Eurasian and African syngens mate with North American corresponding syngens it could be assumed that they evolved in the Americas before they crossed Bering Strait. Then they could have spread thruout Eurasia and Africa, 2 of them (4 and 6) crossing into England, which had a continental connection in the past (20).

It is interesting to note that syngen 3, 4 and 6 are cold strains. They can survive, and 4 and 6 will even grow, at 6 C or less. Only a few strains in syngen 2 can barely tolerate these low temperatures. This may explain why this syngen was not found in Europe; it could not withstand the low temperatures of northern latitudes, a necessity if it were to cross the Bering Strait land bridge. The only other American syngen that tolerates low temperatures is 8, which also was not found in Europe. Perhaps factors other than low temperature tolerance such as sparse distribution accounted for this failure. It is impossible to say that low temperature tolerance is the one character that was responsible for 3, 4 and 6 spreading into Eurasia but it could have been a contributing factor.

C. *The South Pacific Islands and Australia*: The pathway taken by *T. pyriformis* in getting to Australia was most likely from Southeast Asia, island-hopping like the monotremes and marsupials. There are records of monotremes from late Triassic or early Jurassic and for marsupials from late Cretaceous or early Tertiary (32). *T. pyriformis* may have crossed Bering Strait during the early Tertiary Period when there was much faunal interchange. Syngens 3, 4 and 6, any one or all 3, could have reached Australia during this period and subsequently evolved into syngens 11 and 12 which are the only ones that were found on this continent.

Certain similarities exist between the European and Australian syngens. Strains from all of the syngens, except 12, are cold strains and they all have similar refractory periods.

However, both syngens 11 and 12 have no immaturity period which suggests that they are more closely related to syngen 4 than 3 or 6.

It is likely that *T. pyriformis* has been in Australia for a long time, probably antedating man. Had it been carried to this continent by man it does not seem reasonable that such distinct syngens as 11 and 12 could have evolved in so short a time. Moreover, representatives of one or the other syngen, or both, should have appeared in some of the Eurasian collections had they evolved on the continent.

How *T. pyriformis* traversed thousands of miles of ocean and became established on tiny Pacific islands is an interesting question. If it is assumed that this ciliate cannot adapt to sea water and does not form resistant cysts, then vast distances must have been spanned by mechanical means. From laboratory experience one may conclude that this ciliate does not produce cysts, nor can it survive in sea water. In nature, however, there is always the possibility it may do both. In only one case, an estuarine *T. pyriformis* clone survived in both sea and fresh water (15). Cysts have been reported in some cultures in syngen 2 (19). These are isolated cases and judging from our experience in handling thousands of cultures in the laboratory, it is unlikely that they constitute a part of the normal life cycle. The following discussion will ignore either possibility as important in the dispersal of *T. pyriformis* It will be assumed that dispersal to these remote islands was accomplished by mechanical means such as massive air disturbances (hurricanes) and by man during his migrations when water was transported.

There is no question about the possibility of ciliates being carried along with hurricanes, but no records show that the pathways of violent wind disturbances follow those that must have been taken by *T. pyriformis* as it moved from the Americas westward to Hawaii and Fiji. On the other hand, there is some evidence that this ciliate could have been carried by man as he migrated to the Pacific Islands from South America.

It has already been pointed out that syngen 9 inhabits South and Central America, Hawaii and Fiji. Representatives from these localities all interbreed, indicating that they have close affinities and probably have not been separated for any great period of time. Moreover, both Hawaii and Fiji are "young" islands by geologic time standards. The Hawaiian Islands may possibly date back as far as Cretaceous times (7). Most of the rocks making up the Fiji Islands are Tertiary, laid on older bases. Fossils from Fiji also date back to Tertiary times (22). Therefore, animal life reaching these islands must have come at some later time; perhaps many came much later. Since one principal way protozoa could be transported such great distances was in fresh water carried in containers and since man is the only animal that is capable of making long voyages while carrying his drinking water with him, one might conclude that human migrations westward may have played a part in distributing this protozoon to the Pacific Islands. That such voyages did occur is supported by botanical evidence such as the early introduction of the American potato into Polynesia (24, 25). There is strong evidence that these Pacific Islands were not inhabited until approximately 1,500 years ago (4) which would mean that *T. pyriformis* probably arrived since that time.

The available data on syngen 9 seem to fit the hypothesis that the Pacific Islands were populated with this ciliate when it was carried by man in his migrations westward from South and perhaps Central America. If these protozoa had migrated eastward from Asia, they should still exist in Eurasia. They were not found in the European collections which were sufficiently numerous to include them if they were there. Here again lack of data rules out the possibility of drawing any conclusions because the Asian collections were too small to be representative. The free interbreeding of the Pacific Island syngen 9 strains with those from the mainlands of Central and South America is good evidence that they invaded the islands only recently. Moreover, only mating type III was found in both Fijian and Hawaiian waters which means that perhaps only one mating type made the trip. One cannot rule out the possibility of missing other mating types, however, since the collections were limited on both islands.

Based on limited evidence it seems that geographic isolation was primarily responsible for the numerous syngens that we see today. Having been isolated for long periods of time the present syngens have evolved genomes which are only compatible within each syngen. In other words the same isolating mechanisms that have given rise to new species in other organisms have operated in similar fashion in this protozoon.

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Amoeboflagellate Transformations and the Gibbs-Donnan Ratio*

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SYNOPSIS. A number of protozoa may have amoeboid, flagellated, or intergrade forms. At the present time several mechanisms have been proposed for inducing the formation of each of the above forms, but a definite triggering mechanism has not been elucidated. However, some change in the environment precedes the transformation of the cell from one form to another.

Jahn (1962) and Czarska (1964), respectively, correlated ciliary reversal and water expulsion vesicle activity with alterations in the ionic environment. In both cases the processes involved are correlated with changes in the Gibbs-Donnan (G-D) relationship rather than direct ratios or molarities. It seems reasonable to assume that additional environmentally induced phenomena may also be based on changes in the relationship.

The assumption is here made that an amoeboid cell, possessing the necessary genetic and physiologic potentials, can respond to certain changes in its environment by enflagellation.

The following hypothesis is being considered: a change in the environment that increases the relative concentration of associated divalent cations is perhaps one of the main triggers for amoeba-to-flagellate transformations. Thus, in accordance with the G-D theory, this transformation would be expected to occur when a given ionic environment is diluted. In addition, the transformation is discussed in relation to pH, population density, and other environmental parameters that alter the Gibbs-Donnan ratio.

THE amoebo-flagellates are a group of eucaryotic cells capable of changing from an amoeboid to a flagellate form and vice versa. Representatives of this group include *Naegleria gruberi, Tetramitus rostratus, Dimorpha floridanis, Gigantomonas herculea, Heteramoeba clara* (28) and *Didymium nigripes* (15). The ability of the amoeboflagellates to exist as 2 distinct phenotypes, with or without intermediate or intergrade forms, has made this group a popular tool in studies concerned with cell differentiation and/or morphogenesis. However, the stimulus that induces the amoeba-to-flagellate transformation (AFT) has remained enigmatic.

Numerous papers elucidating the stimuli which can induce the AFT have appeared. The proposed stimuli were summarized by Willmer (29), as given in Table 1. The stimuli listed by Willmer have remained essentially unchanged by subsequent investigation and there is apparently no reason to assume that only a given stimulus is responsible for the transformation. Indeed, it is readily obvious that all of the stimuli listed in Table 1 are significant parameters of any biological system. In addition, the interrelationships of the majority of these stimuli has never been evaluated in terms of the AFT.

If we assume that the AFT requires the amoeba to possess the necessary genetic ability to respond in conjunction with the correct physiological state, the list of stimuli may be reduced to 2 categories, namely, mechanical effects and physiochemic changes. Both stimuli can alter the physical state of an amoeboid cell and may be affected and reflected as changes in cationic ratios. The assumption that the AFT is due to an environmental change is inherent in Table 1. Thus, the list of stimuli is reduced to a common basis and all that is needed is a mechanism whereby the amoeboid cell is affected by a significant

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