It will be evident from the foregoing outline that the vascular-structures of the seedling are not constant but are highly variable, even within genetically very homogenous material. It is quite possible, therefore, that anatomical investigations based on but a few sections for each species might lead to erroneous results. Seedlings differing in external form are differentiated in their internal anatomy. Such differentiation is evident not only in mean number of bundles but in the degree of variability in bundle number. Thus, in normal seedlings the variability is higher in the hypocotyl than in the epicotyl, whereas in seedlings with three cotyledons and three primordial leaves just the reverse is true. The external form and the internal structure of the seedling are highly but not perfectly correlated. Finally in both normal and variant seedlings, the number of vascular elements of the several regions of the seedling are correlated in very different degrees; the correlation between some is high; between others it is practically wanting.

Papers to appear in the *American Journal of Botany* may be consulted for details.

[1] The values given in each case are the maximum and minimum constants for the lines investigated. For the trimerous and dimerous seedlings all the averages are based on five lines. For the hemitrimerous seedlings the averages for primary double bundles and intercalary bundles are based on five lines while for the mid-region of hypocotyl and the mid-region of epicotyl they are based on six lines. Data for number of root poles are available for only three lines.

[2] Because of the extreme rarity of hemitrimerous seedlings it is not feasible to discuss variability of bundle number in this group.

THE PRESENT STATUS OF THE LONG-CONTINUED PEDIGREE CULTURE OF PARAMECIUM AURELIA AT YALE UNIVERSITY

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Communicated by R. G. Harrison, December 30, 1920

As a matter of record it seems advisable to bring up to date and summarize the chief results derived from the writer's main pedigree culture of Paramecium aurelia.

This culture, designated Culture I, was started on May 1, 1907, by the isolation of a "wild" individual which was found in a laboratory aquarium.[1] The original specimen was placed in about five drops of culture fluid on a glass slide having a central ground concavity, and when this animal had produced four individuals, each of these was isolated on a separate slide to form the four lines of the culture. The four lines have not been kept distinct from one another throughout the work, but have been replenished by cells from one of the sister lines when, through accident or otherwise, one or another of the lines has become extinct.

The culture has been maintained by the isolation of a specimen from each of these lines practically every day, with the exception noted below. The number of divisions in each line has been recorded at the time of isolation and the average rate of these four lines has again been averaged for varying numbers of days (5, 10, or 30 days) as the exigencies of the different experiments demanded. These data have afforded the graphs of the division rate. Permanent preparations have been preserved from time to time for the study of the cytological changes during the life history. During the first eight months of the work the culture medium consisted of infusions of hay and fresh grass, but from February, 1908, to the present time various materials collected from ponds, swamps, etc., have been employed. The infusions were thoroughly boiled to prevent the contamination of the pure culture with foreign strains of Paramecium. In short, the cells of the four lines of the culture to-day are direct lineal descendants by division of the single animal isolated in 1907.

The object of starting the culture was to determine whether Paramecium can reproduce by division indefinitely without recourse to conjugation. Throughout the work the possibility of conjugation in the four lines of the culture has been precluded by the almost daily isolation of the products of division. Accordingly its continued life and health has long since justified the conclusion that conjugation, involving syncaryon formation, is not, as previously generally maintained, a sine qua non for the continued life of Paramecium, in particular, and, presumably, of Infusoria in general. At the completion of the

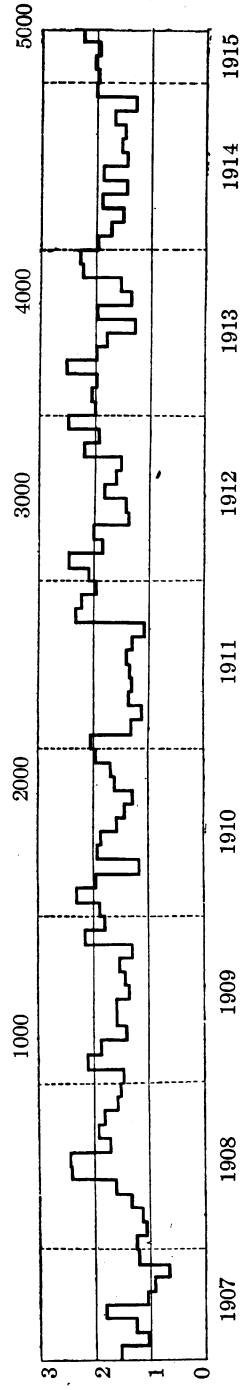

FIG. 1

Graph showing the rate of reproduction of the pedigree race (I) of Paramecium aurelia during the first eight years of culture. The ordinates represent the average daily rate of division of the four lines of the culture, again averaged for each month. The vertical broken lines indicate the limits of the calendar years. The figures 1000, 2000, etc., represent generations and are placed above the periods in which they were attained.

culture's first five years of life, during which over 3029 generations were attained, the writer stated that "the organisms of the present generation are in as normal morphological and physiological condition as the original 'wild' individual isolated to initiate the culture. This study has indicated that, under favorable environmental conditions, the protoplasm of the cell originally isolated possessed (at least) the potentiality to produce similar cells to the number represented by 2 raised to the 3,029th power, or a volume of protoplasm not less than 10^{1000} times the volume of the Earth. I believe this result indicates beyond question that the protoplasm of a single cell may be self-sufficient to reproduce itself indefinitely, under favorable environmental conditions, without recourse to conjugation....."

As a matter of fact organisms taken from this culture of Paramecium have shown but little tendency to conjugate. From time to time experiments to induce conjugation have been carried out in mass cultures started from the pedigree lines. The first successful experiment occurred in December, 1913, at about the 4100th generation.[2] The next epidemic of conjugation in mass culture occurred June, 1920.

Careful studies of the rate of division of sub-lines derived from the main lines and bred under the most constant environmental conditions revealed the fact that there are inherent, normal, minor, periodic rises and falls of the fission rate due to some unknown factor in cell phenomena. These were termed Rhythms.[3] In a search for the underlying factors of rhythms a complicated internal nuclear reorganization process was discovered and named Endomixis.[4] This process involves the periodic formation of a complete new nuclear apparatus by a definite sequence of normal morphological changes which simulate those occurring in conjugation. Endomixis, in essence, consists of a gradual disintegration and absorption of the macronucleus in the cytoplasm. Simultaneously a multiplication of the micronuclei is in progress. Certain of the resulting micronuclei degenerate while the remaining one or two form the new macronuclear and micronuclear apparatus of the cell. This results in the

YEAR	GENERATIONS	DAILY AVERAGE DIVISION RATE
1	452	1.24
2	690	1.89
3	613	1.68
4	612	1.67
5	662	1.81
6	692	1.89
7	671	1.84
8	679	1.86
Average for 8 yrs.	634	1.74

reorganization of the cell (endomixis) without the coöperation of two animals involving syncaryon formation (amphimixis) as in conjugation.

After endomixis was worked out, it seemed unnecessary to continue longer the main pedigreed culture and accordingly on May 1, 1915, at the 5071st generation, the experiment was *formally* considered as closed.

The record of divisions for each year of the life of the culture to May 1, 1915, may be tabulated as shown in the preceding table.

Thus for eight years the culture averaged about fifty generations per month. However, at the formal termination of the experiments in 1915, I was reluctant to discard the race and was tempted to keep it under control but without such exacting daily observation and recording of the daily division rate. In this manner it has been maintained up to the present (December, 1920). But, from time to time, thirty-day tests have been made of the vitality of the race under the former rigid culture conditions, and in each case the same general average division rate has been revealed as during the first eight years of its life. For example, during December, 1915, 52 generations; during December, 1917, 56 generations; and during November, 1920, 54 generations were attained. On the basis of such tests it is fair to make the exceedingly conservative estimate of 600 generations attained each year since May 1, 1915. This would give, in round numbers, 8400 generations attained by the culture during the $13^1/_2$ years of its life to date.

Thus the conclusion is still justified that conjugation is not a necessary phenomenon in the life history of Paramecium aurelia under favorable environmental conditions. But there is an internal reorganization process (endomixis) which occurs periodically. Whether endomixis is a necessary factor for the continuance of the race is another question—a new question which has been raised by these studies, and is now under investigation.[5]

[1] Woodruff, L. L., "The life cycle of Paramecium when subjected to a varied environment," *Amer. Naturalist*, **42**, 1908. "Two thousand generation of Paramecium," *Archiv f. Protistenkunde*, **21**, 1911.

[2] Woodruff, L. L., "So-called conjugating a non-conjugating races of Paramecium," *J. Exper. Zool.*, **16**, 1914.

[3] Woodruff, L. L. and Baitsell, G. A., "Rhythms in the reproductive activity of Infusoria," *J. Exper. Zool.*, **11**, 1911; "The temperature coefficient of the rate of reproduction of Paramecium aurelia," *Amer. J. Physiol.*, **29**, 1911.

[4] Woodruff, L. L., and Erdmann, R., "A normal periodic reorganization process without cell fusion in Paramecium," *J. Exper. Zool.*, **17**, 1914.

[5] Woodruff, L. L., "The problem of rejuvenescence in protozoa," *Biochem. Bull.*, **4**, 1915; "The influence of general environmental conditions on the periodicity of endomixis in Paramecium aurelia," *Biol. Bull.*, **33**, 1917.