

those produced by x-irradiation, the above result and further experiments producing mutations with neutrons may lead to new conclusions regarding the interaction of radiation ions with genes and hence of the size and structure of the gene.

It is interesting to note that the ratio of effectiveness (5 or 6 to 1) for the effects on mature sperm of neutron as compared with x-irradiation is comparable to that for carcinoma cells (5 or 6 to 1), and actively sprouting seedlings (5 to 1), but greater than that for *Drosophila* eggs (2.1 to 1), for fern spores (2.5 to 1) and normal mice (4 to 1), all of which were dosed by the same method. The action on mature sperm measured in the experiments here described is one in which chromosome changes probably are much the most important element. It may be possible by further study of ratios of effectiveness to determine the cell element which first breaks down under irradiation in any given case.

Results obtained in later generations will be reported in a subsequent paper.

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SEX, SEX INHERITANCE AND SEX DETERMINATION IN *PARAMECIUM AURELIA*

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Paramecium has long appeared an outstanding example of the absence of sexual differentiation in individuals that nevertheless conjugate. In a certain race of *Paramecium aurelia*, however, I have discovered that there is functional sex diversity. Two classes of individuals exist. Members of different classes unite for conjugation; members of the same class do not.

This relation was discovered in pursuance of the author's study of the

genetic results of endomixis. It was found that after endomixis, in certain cases the numerous descendants of a single individual that has undergone endomixis will not conjugate together, but that they will under the same conditions conjugate with descendants of certain other exendomictic individuals. Following this clue, the entire stock was found divisible into two sex classes, and the phenomena were unraveled as set forth in the following.

Different races of *Paramecium aurelia* show remarkable diversities in the sexual phenomena, so that I shall first give an account of them in the race *S* in which they were discovered, then mention some of the different relations in other races.

Race S.—Race *S*, originally from a pond of Cold Spring Harbor, Long Island, was obtained from Drs. Schaeffer and Wichterman, of Temple University.

Sexual Behavior.—When cultures derived from single individuals without the intervention of endomixis are mixed together, in some of the mixtures the individuals immediately clump together in clusters, from which they later emerge as conjugated pairs. In other mixtures no clumping or conjugation occurs. If samples from a number of such cultures are paired off in all possible combinations, it is found that they are divisible into two groups. Any culture of the one group will form clusters and conjugants when mixed with any culture of the other group. But no sexual reaction (clumping or conjugation) occurs when any two cultures of the same group are mixed together. The two sex classes thus revealed will be called for convenience sex I and sex II. They do not differ in size or in any visible respect.

When many individuals of the two sexes are placed together (at periods when they are ripe for conjugation), a very striking reaction occurs. United pairs are formed almost instantaneously. In less than a minute there are clusters of three or four individuals. Within five minutes practically all the individuals are in clusters, each containing from two to forty animals. The clusters persist for forty-five minutes to an hour, during which time the definite pairing takes place. Then the clusters separate into pairs of conjugants, which remain united (at room temperature) for about eight hours.

Final conjugation appears to occur only between individuals of opposite sex type, though during the cluster formation individuals of the same sex type may cling together for brief periods. This is demonstrated by introducing a single individual of one sex into a drop containing many individuals of the other sex. A cluster of several individuals is formed, from which finally emerges only a single pair of permanent conjugants. But single individuals that separate from the cluster may join themselves temporarily to others, necessarily of the same sex type, so that for a short time two, three or four pairs may be present. In a few minutes all of these pairs separate except one. That this remaining pair includes the single individual of the intro-

duced sex may be demonstrated in the following way. For introduction an individual is chosen that is larger or smaller than usual, so that it can be recognized. The single final pair is always found to contain this individual; its mate is necessarily an individual of the other sex type, so that actual conjugation occurs only between individuals of different sex.

From the facts just described it appears that after contact with an animal of the other sex, an individual can effect brief union with other individuals of its own sex. Attempts were made to induce in this way completed conjugations between individuals of the same sex, but in the 20 experiments carried out in this way this never happened; the two individuals of the same sex always separated without completing conjugation.

Cluster formation and pairing are seemingly not due to the action of certain substances in the fluids in which the two sexes are growing, for they are not induced by introducing into colonies of one sex fluid from colonies of the other sex. Filtrates and centrifugates (lacking animals) from large dense cultures of one sex are completely ineffective when introduced into cultures of the other sex. Apparently the actual presence of individuals of the other sex is necessary for inducing the sexual reaction.

There are certain periods in the life history when the sexual reactions above described do not occur. This is the case for about a week after conjugation. Then for a day or two before the complete reaction is producible, a small number of conjugant pairs can be obtained by keeping the animals for six to twelve hours in mixtures with the other sex. This brief period of delayed and weak reactivity is followed by a period during which the typical complete reaction occurs, and this lasts until the stock begins to undergo endomixis. During endomixis the organisms always completely lose their sex reactivity. They regain it in full strength within three days after the climax of the endomictic process.

Except for these brief periods in the life history, and during fission, the animals appear capable, under proper physiological conditions, of immediate sexual reaction at any time. The proper physiological condition consists simply in the animals not being overfed. Individuals taken directly from daily isolation lines in which there is an excess of food will not react at once; but if such a culture is left unchanged for two or three days, immediate sexual reaction will occur when the other sex is introduced. However, starvation is not required; indeed, the animals react best when they contain many food vacuoles and appear in excellent nutritive condition.

Inheritance of Sex.—(a) *Fission.* In the race *S*, the sex type is strictly and invariably inherited in reproduction by fission, provided endomixis or conjugation does not intervene. All the numerous products of fission from an individual of either sex are of the same sex as the progenitor.

This is demonstrated in two ways. (1) If a single individual of sex I is

allowed to propagate and form a large mass culture, clumping and conjugation never occur in this culture even if it is subjected to environmental conditions known to be favorable for the induction of conjugation. Moreover, if samples from this culture are mixed with other cultures known to be of sex I, no clumping of conjugation occurs; but if samples are mixed with cultures known to be of sex II, cluster formation occurs at once and is followed by conjugation. Likewise, large cultures developed from a single individual of sex II never conjugate among themselves or when mixed with other cultures of sex II, but the conjugation reaction occurs at once when mixed with animals of sex I. (2) If an individual of sex I gives rise to a daily isolation line and the surplus animals from each day's culture are later tested as to sex by placing some with sex I animals and others with sex II animals, clumping and conjugation never occur in the former, but always in the latter. The converse occurs in the daily surplus cultures from isolation lines begun with an individual of sex II.

This constancy of sex makes it possible to keep on hand at all times large cultures of either sex for use in ascertaining the sex of new lines of descent. Tests are made by placing some animals from the new line in a drop with animals from a culture known to be of sex I, and others in a drop from a culture known to be of sex II. In every case, clumping and conjugation occur in one of the mixtures, but not in the other. The new line is shown to be of the same sex as the tester with which it fails to conjugate and of sex opposite to that of the one with which it does conjugate. This method of testing was employed in the experiments described in the following paragraphs.

(b) *Conjugation.* After conjugation, as before set forth, there is a period of about a week during which sex reactivity disappears. If the progeny of different exconjugants are kept separate, it is found that when the sexual reactivity reappears, certain of the exconjugants have given origin to descendants all of which are of the same sex as their progenitor before conjugation; others to descendants that are of the opposite sex from their progenitor before conjugation, while still others give rise to one line of descent that is of sex I and one line that is of sex II.

By employing for mating large well-fed individuals of sex II and small poorly fed specimens of sex I, it was possible to recognize the two sexes before, during and for some time after conjugation. Of 22 exconjugants that had been of sex I before conjugation, 5 gave rise to progeny of sex I only, 7 gave rise to progeny of sex II only and 10 gave rise both to some of sex I and some of sex II. Of 25 exconjugants that had been of sex II before conjugation, 4 gave rise to progeny of sex II only, 3 gave rise to progeny of sex I only and 18 gave rise to some of sex I and some of sex II.

In those of the exconjugants that gave rise to both sexes, the separation of the two sexes occurred at the first fission after conjugation. All the de-

scendants of one of the products of this fission were of sex I, while all from the other product were of sex II.

This was ascertained by following and testing separately the descendants of the eight individuals produced in the first three fissions after conjugation by each of 23 exconjugants (thus 184 lines in all). From three of these exconjugants all the eight lines were of sex I; from eight others the eight lines were all of sex II. From each of the remaining 12 exconjugants, the four lines from one product of the first fission were of sex I, the four from the other product of the first fission were of sex II. Thus no separation of sexes occurred after the first fission following conjugation.

These results were confirmed by examination of many large cultures each derived from one of the products of the first fission of a single exconjugant. Though each such culture contained thousands of individuals, conjugation never occurred in any of them (so long as endomixis had not taken place). If the cultures had contained both sex types conjugation would have occurred in them, as often does occur when the cultures are made up of all the descendants of a single exconjugant. Thus in any culture derived from but one of the products of the first fission all the individuals were of the same sex. Separation of the two sexes in the descendants of an exconjugant occurs at the first fission or not at all.⁷

Therefore, in order to discover the rules for the inheritance of sex, one must deal with the descendants of the products of the first fission after conjugation as units. These rules were studied in several matings between members of the same or of different clones of race *S*. All gave the same type of results, so that they will be considered together.

Altogether, the descendants of 316 individuals that were products of the first fission after conjugation were carefully tested as to sex. Of these, 158 were of sex I, the other 158 of sex II, giving a ratio of 1:1. In 139 exconjugants the descendants of both products of the first fission were tested. Of these, 35 exconjugants gave both sets of descendants of sex I, 70 yielded one set of sex I, the other of sex II and 34 gave both sets of sex II. The ratio for the three types of distribution is therefore 1:2:1.

For 56 pairs, the two sets produced by each exconjugant were tested, giving thus four sets for each pair. Of the 56 pairs, but one had all four sets of type I. Eighteen gave three sets of type I, one set of type II. Twenty-one gave two of type I, two of type II. Thirteen gave one of type I, three of type II and three gave all four sets of type II. These ratios 1:18:21:13:3 are fairly close to a 1:4:6:4:1 distribution (for perfect agreement 56 pairs would have given 3.5:14:21:14:3.5).

These ratios show that the distribution of sexes among the products of the first fission of exconjugants is a strictly chance one. There is no greater tendency for the two sets of individuals from a single exconjugant, or for the four from a pair, to be alike in sex than for any two or four taken at

random. The two parents are of course of different sex; their four sets of progeny consist of the two sexes in chance combinations. The ratios are identical with those for inheritance in higher organisms, in which the characteristics are dependent on genes in the chromosomes.

(c) *Endomixis*. At endomixis, as at conjugation, sex differentiation occurs. The individual of a given sex that undergoes endomixis produces descendants that may be of either sex, or part of them of one sex, part of the other. That is, some give rise to progeny all of which are of the same sex as before endomixis; others give rise to progeny all of which are of the sex opposite to the one existing before endomixis; the rest give rise to one line of descent of sex I and one line of descent of sex II. In this last case, the separation into the two different lines of descent occurs at an early fission after the climax of endomixis, probably the first. The exact fission at which this separation occurs and the proportions of endomictic individuals showing each of the three types of relations just set forth are now under investigation in this laboratory. Maintenance of sex, change of sex and segregation into two sex types occur at every endomictic period and in every one of the many clones of this race thus far investigated. Attempts to obtain "pure" lines which do not change sex at endomixis, by selecting after each of five successive endomixes individuals of the same sex as existed before endomixis have failed; both the selected line of sex I and the selected line of sex II continue to produce both sexes at endomixis.

Sex Determination.—It is evident that the method of inheritance above described indicates that sex determination is nuclear, as in most other organisms. That the macronucleus rather than the micronucleus is differentiated as to sex appears probable, since studies of amacronucleate and amicronucleate ciliates indicate that the macronucleus controls the physiological processes of the organism. Moreover, at endomixis and conjugation the macronucleus disappears, and at these times the sex reactivity is lost. Two new macronuclei⁷ are then formed from the micronuclei in each exconjugant and in each endomictic individual and two sexually different lines of descent may develop from such individuals. These two macronuclei are distributed to the two products of the first fission; sex likewise may segregate at the first but not at a later fission after conjugation, and possibly also after endomixis. Thus, all individuals that contain descendants of one macronucleus are of the same sex.

The ratios in which the two types of macronuclei appear indicate that the difference between them involves but one pair of chromosomes. How these chromosomes are disposed cannot yet be demonstrated, but a few of the more probable possibilities may be briefly pointed out.

(1) The two sex chromosomes may be alike, but macronuclei of one sex may contain two of them while macronuclei of the other sex contain but

one. This could be brought about by the loss of one sex chromosome in the development of half the macronuclei. In this case, all micronuclei would be homozygous for sex.

(2) The two sex chromosomes may differ, one member of the pair occurring in macronuclei of one sex, the other member in macronuclei of the other sex. The macronuclei would thus be haploid with respect to the sex chromosomes. If so, the micronuclei (known to be diploid in other ciliates) must be heterozygous for sex. This heterozygous condition may be maintained in either of two ways, depending upon when segregation of the sex chromosomes occurs during conjugation: (a) Segregation of the sex chromosomes at the third maturation division. This would result in different sex chromosomes in the two pronuclei formed in each conjugant. If the pronucleus that contains a particular one of these chromosomes regularly transforms into the migratory pronucleus, the heterozygous condition will be perpetuated. (b) Segregation of the sex chromosomes at the first or second maturation division. This would result in four haploid nuclei with one sex chromosome and four with the other one. Of these eight, only one persists to form the pronuclei. If the one that persists always contains the same sex chromosome as that in the macronucleus (or if the other one regularly persists), then the two pronuclei in one conjugant of each pair must contain one sex chromosome, and the two pronuclei in the other conjugant would contain the other sex chromosome. Heterozygosity of the micronuclei would thus be maintained.

A decision among the possible chromosome mechanisms must await a fuller knowledge of the cytology and genetics of conjugation and endomixis.

General.—The foregoing account presents the situation in the single race *S* of *Paramecium aurelia*. Examination of other races of this species indicates that the situation differs in a marked way in different races. Four other races have been examined in comparison with race *S*. In three of these—Woodruff's long-lived Yale race, and two races from different localities in Maryland—the organisms do not form clusters or conjugate when mixed with either sex of race *S*. In a fourth, the race *R*, reported on in numerous contributions from this laboratory, there appears to be differentiation into the same two sexes as in race *S*, but conjugation nevertheless occurs among the descendants of products of the first fission soon after conjugation or endomixis, as set forth by Sonneborn.¹ Further study of this race is now in progress.

A number of points in Protozoan literature are touched by the results here set forth; a few of these may be briefly mentioned.

(1) The idea that sex differentiation in such ciliates as *Paramecium* is confined to the two pronuclei formed at conjugation obviously does not hold for stocks *S* and *R* of *P. aurelia* in which the individuals themselves are sexually differentiated.

(2) The Chattons² and Zweibaum³ have maintained that conjugation is entirely determined by environmental conditions. Others (Sonneborn and Cohen⁴ and Sonneborn and Lynch⁵) have shown that genetic factors are also involved. The present results show the overwhelming importance of genetic factors in some stocks.

(3) Calkins and Gregory⁶ long ago attempted to show that the descendants of the four products of the first two fissions after conjugation in *P. caudatum* (where four instead of two macronuclei are formed in each exconjugant) were genetically diverse. The present work confirms this principle in a striking way.

It may perhaps be said that with the present work the genetics of *Paramecium* enters the quantitative and predictable stage, with tools and methods of analysis which should lead rapidly into a systematic, coherent body of knowledge in close touch with the rest of genetic science.

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⁷ Further work has shown that in a small proportion of the exconjugants sex may segregate at the second fission after conjugation; and this has been correlated with the fact that in a similar proportion of the exconjugants three or four instead of two macronuclei are formed.

THIAZOLE AND THE GROWTH OF EXCISED TOMATO ROOTS

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We have reported earlier¹ that excised tomato roots do not grow in a nutrient solution of mineral salts and pure cane sugar. They grow satisfactorily, however, in the same solution to which dried brewer's yeast is added. We have found that the substitution of crystalline vitamin B₁ for the yeast permits growth to occur and we have grown excised tomato roots in a nutrient solution consisting of mineral salts, cane sugar and crystalline vitamin B₁ for over seven months through seven subcultures. It appears that unlimited growth of excised tomato roots is possible in this medium. We have, therefore, been able to define more exactly the nutrient requirements of tomato roots by substituting for the numerous and varied constituents of brewer's yeast a single definite chemical compound, namely vitamin B₁, and thus to prepare a synthetic solution of known con-