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THE INHERITANCE OF SEX AT ENDOMIXIS IN PARAMECIUM AURELIA[10]

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Recently, Sonneborn[1] reported the discovery of a sexually differentiated race of *Paramecium aurelia*. In this race, *S*, every individual is of one of two sexes, called by him sex I and sex II; and the vegetative progeny of any individual are all of the same sex as the progenitor. When cultures of different sex are mixed, the individuals unite at once for conjugation; but when cultures of the same sex are mixed, no conjugation occurs. Sonneborn reported in detail the inheritance of sex at conjugation but gave only preliminary, qualitative results on its inheritance at endomixis. The present paper gives a precise and quantitative analysis of the segregation, inheritance and determination of sex at endomixis, and of certain related matters.

The inheritance of sex at endomixis was examined in three groups of individuals of the race *S* reported on by Sonneborn.[1] Each group consisted of a number of daily isolation lines descended vegetatively from a single individual. The three original individuals were closely related, but two of them were of sex I and one was of sex II. Samples from all the lines of the three groups were stained daily according to the method of Sonneborn[2] to determine when endomixis occurred. After each period of endomixis in each of the groups, all but one line was discarded; and a new set of lines was expanded from the one retained. To perpetuate the group a line was always selected of the same sex as the original progenitor. In this way, there were always maintained throughout the course of the work two groups of sex I and one group of sex II.

Segregation of Sex.—Sonneborn[1] found that some endomictic individuals give rise to two lines of descent differing in sex and that this segregation of sex occurs soon after the beginning of endomixis. The first question for analysis, therefore, was to ascertain the precise point at which this segregation occurs. As endomixis is a process which extends through several fissions, it was necessary to establish a definite point of reference for the study.

A brief description of the process will be necessary to make clear the subsequent account.

According to Woodruff and Erdmann,[3] endomixis begins with the gradual disintegration of the macronucleus. After the stage known as climax, there may or may not be a fission. In either case in each of the one or two individuals formed, two products of the micronuclei transform into new macronuclei through preliminary growth stages known as anlagen. These two anlagen are then segregated into separate individuals at the next fission, thus restoring the normal number of one macronucleus. In the subsequent fissions, the new macronuclei divide; and the fragments of the old macronucleus are irregularly segregated. Eventually the latter become resorbed, but they are visible in diminishing numbers for several fissions.

In order to ascertain the point at which sex is segregated, it was necessary to obtain and recognize those individuals in which had occurred the complete disintegration of the old macronucleus and the formation of the two anlagen of the new macronuclei. This was accomplished in the following way. Small mass cultures were set up consisting of the surplus animals from the isolation lines of the group to be tested. Endomixis was then induced by the method described by Sonneborn.[4] Observations were made at least every twelve hours to be sure that no conjugation had occurred. In no case was it ever found. At various intervals, a number of animals from the culture were stained with aceto-carmine to determine whether endomixis was occurring and if so, what stage the animals were in. When the culture was found to contain a large number of animals with two anlagen, some in earlier stages, some non-endomictics, and a very few or none with one anlage—i.e., after the first division after the formation of the anlagen—it was considered suitable for selecting animals in the two-anlagen stage. At this time, many of the animals with two anlagen are larger than most (or all) non-endomictics, and they may further be distinguished by their clear and narrow anterior end. Animals with these characteristics were chosen; and in every experiment some of those chosen, both before, during and after the selection of animals for the experiment, were stained in order to be sure that the selection was made accurately. That it was is shown by the fact that of 171 animals so chosen and stained 157 had two anlagen, 3 were in climax, 2 had only one anlage and 9 were non-endomictics. Those in the two-anlagen stage and probably those in climax were those required for the study. The non-endomictics were always eliminated by discarding those lines in which no fragments of the old macronucleus were found in one of the products of the second or third fissions. This method assures the selection of groups in which practically all the animals are in endomixis just prior to the first fission after the formation of the anlagen since, as the data show, not more than 3% of these groups were animals which were in endomixis prior to the formation of the anlagen or after the first fission. This error is too small to appreciably affect the results.

The cultures to be tested for sex were obtained by allowing each of the selected endomictic individuals to multiply in from 4 to 5 drops of culture fluid until many animals were present and the food supply had begun to decline. Each culture was then tested for sex type by adding some of the individuals to each of two groups of animals: one group known to be sex I; the other, to be sex II. In nearly all cases conjugation occurred in one of these cultures only. The few exceptions to this are now under investigation. The sex of the animals tested was, of course, the opposite of that of the animals with which conjugation occurred.

Two extensive experiments were performed to determine the fission at which sex segregates at endomixis. In these experiments the two products of the first fissions of 181 individuals in the two-analgen stage of endomixis were isolated, and their progeny later tested for sex type. The two cultures from 96 of these individuals proved to be of the same sex, the two cultures from the remaining 85 individuals were of opposite sexes. Thus sex segregates at the first fission after the formation of the anlagen in approximately one-half the endomictic individuals.

In order to determine whether sex also segregates at a later fission, 353 products of the first fission after the formation of the anlagen were allowed to divide a second time; and, in each case, the two products of this second fission were isolated, and their progeny later tested for sex type. In 349 of the 353 cases, the two cultures thus produced proved to be of the same sex. In two of the other four cases, one of the cultures was of sex I; and the other formed pairs when mixed with animals of either sex. In the remaining two, one culture was of sex II; and the other reacted with both sexes. Three of the four exceptional cultures also contained conjugants at the time they were tested for sex type. None of these four cultures was tested for sex type sooner than six days after endomixis. Whether these four cultures actually represent exceptions to the general rule that the segregation of sex occurs only at the first fission after the formation of the anlagen or whether they can be explained on some other basis remains for a more thorough investigation to answer. It may therefore be concluded that sex never, or very rarely, segregates at a later fission than the first after the formation of the anlagen.

Sex Ratios.—The two sexes were produced in three different ratios in the six experiments that were performed. In four of the experiments the following numbers were obtained:

EXPERIMENT	1	2	3	4	TOTAL
Sex I	71	26	66	88	251
Sex II	63	21	55	94	233

These results are fairly close to the exact 1:1 ratio of:

EXPERIMENT	1	2	3	4	TOTAL
Sex I	67	$23^{1}/_{2}$	$60^{1}/_{2}$	91	242
Sex II	67	$23^{1}/_{2}$	$60^{1}/_{2}$	91	242

In another experiment 128 individuals of sex I and 63 of sex II were obtained, this being almost exactly a 2:1 ratio (mathematically, $127^{1}/_{3}$: $63^{2}/_{3}$). In a third experiment 63 individuals of sex I and 137 of sex II were obtained, which is close to a 1:2 ratio (mathematically, $66^{2}/_{3}$: $133^{1}/_{3}$).

Sonneborn found three different classes of endomictics: those that give rise solely to sex I; those that give rise to both sex I and sex II; and those that give rise solely to sex II. The same three classes were found in the present work. If random distribution of the two sexes to the two products of the first fission occurs, the proportion of each of these classes produced depends on the ratio in which the two sexes occur. With a 1:1 sex ratio, a 1:2:1 distribution of the three classes is expected; with a 2:1 sex ratio, a 4:4:1 distribution; and with a 1:2 sex ratio, a 1:4:4 distribution. In the four experiments which gave a 1:1 sex ratio, the following numbers of endomictics of the three classes were obtained:

EXPERIMENT	1	2	3	4	TOTAL
All sex I	19	5	14	21	59
Sex I and sex II	31	11	31	45	118
All sex II	13	3	10	22	48

With an exact 1:2:1 ratio, the following would be the numbers:

EXPERIMENT	1	2	3	4	TOTAL
All sex I	$15^{3}/_{4}$	$4^{3}/_{4}$	$13^{3}/_{4}$	22	$56^{1}/_{4}$
Sex I and sex II	$31^{1}/_{2}$	$9^{1}/_{2}$	$27^{1}/_{2}$	44	$112^{1}/_{2}$
All sex II	$15^{3}/_{4}$	$4^{3}/_{4}$	$13^{3}/_{4}$	22	$56^{1}/_{4}$

In the experiments which gave a 2:1 sex ratio, the numbers of endomictics of each of the three classes were 42:40:11, almost identical with the theoretical numbers ($41^{1}/_{3}$:$41^{1}/_{3}$:$10^{1}/_{3}$). In a third experiment, in which a 1:2 sex ratio was obtained, the number of endomictics of each of the three classes were 13:36:46 which is fairly close to the theoretical 1:4:4 ratio of $10^{5}/_{9}$: $42^{2}/_{9}$:$42^{2}/_{9}$. Therefore it can be concluded that the distribution of the two sexes to the products of the first fission after the formation of the anlagen is purely random.

Change of the Sex Ratio in a Single Line of Descent.—In two of the three groups examined, a different sex ratio was obtained in the second test from that obtained in the first. In one, the ratio changed from 1:1 to 2:1; in the other from 1:2 to 1:1. The two groups had a similar history between the tests. Each went through many vegetative fissions; each went through endomixis, the first once, the second twice; but neither went through conjugation. Therefore the change of ratio must have occurred either at vegetative fission or at endomixis, though neither of these processes necessarily involves a change of ratio since the third group went through both without changing its ratio. However, change of ratio must occur with considerable frequency since it was found in two out of the three groups twice tested.

Sex Determination.—The relations reported here for endomixis and those reported by Sonneborn[1] for conjugation are very similar. In both processes sex is segregated to different individuals at the first fission after the formation of the anlagen; and, at this same fission in both, the two new macronuclei that are formed are also segregated. Thus it is extremely probable that sex is determined by the macronucleus and that two different types of macronuclei can be formed: one determining sex I; and one determining sex II. Thus far sex determination at endomixis is just like that reported by Sonneborn for conjugation in this race. However, the experiments reported here show that after endomixis there are at least three sex ratios while Sonneborn reports only one, the 1:1 ratio after conjugation.

Sonneborn has suggested two possible chromosome mechanisms to account for the facts that he observed. These apply equally well to endomixis when a 1:1 ratio occurs. However, modifications are needed to explain the 2:1 and 1:2 ratios and also to explain the change of ratio. What the chromosome mechanism actually is can only be determined after a more thorough study.

Relation of the Results to Other Problems.—The results reported here have a bearing on several other problems of protozoölogy.

(1) It has been held in some quarters that endomixis is a variable reaction to diverse environmental factors and not a definite process as is conjugation. The preciseness of the ratios and of the segregation of sex as shown by these experiments indicates, on the other hand, that endomixis is quite as regular and exact a process as is conjugation.

(2) Diller[5] contends that much of what has been called endomixis is in reality autogamy or self-fertilization. While perhaps the close similarity of sex inheritance at conjugation and at endomixis seems to indicate that this is the case, actually the present results neither confirm nor contradict this contention but could equally well be explained on the basis of endomixis as described by Woodruff and Erdmann[3] or on the basis of autogamy as described by Diller.[5]

(3) The origin at endomixis of genetic diversities in other characters than sex has been reported by Erdmann,[6] Parker,[7] Caldwell[8] and Sonneborn and Lynch.[9] The fact that within a clone the macronuclei of different products of the first division after the formation of the anlagen at endomixis may differ in their sexual potency makes it seem possible that they may also differ in their potency in relation to other characters, thus explaining to a

degree the origin of hereditary diversities at endomixis. To what extent, if at all, this may be true remains for future investigation to discover.

[1] Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, **23**, 378–385 (1937).

[2] Sonneborn, T. M., *Genetics*, **21**, 503–514 (1936).

[3] Woodruff, L. L., and Erdmann, R., *Jour. Exptl. Zoöl.*, **17**, 425–518 (1914).

[4] Sonneborn, T. M., *Biol. Bull.*, **72**, 196–202 (1937).

[5] Diller, W. F., *Jour. Morph.*, **59**, 11–67 (1936).

[6] Erdmann, R., *Arch. f. Entwmech.*, **46**, 85–148 (1920).

[7] Parker, R. C., *Jour. Exptl. Zoöl.*, **49**, 401–439 (1927).

[8] Caldwell, L., *Jour. Exptl. Zoöl.*, **66**, 371–407 (1933).

[9] Sonneborn, T. M., and Lynch, R. S., *Genetics*, **22**, 284–296 (1937).

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A THIRD LETHAL IN THE T (BRACHY) SERIES IN THE HOUSE MOUSE

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Congenital absence of the tail in a strain of mice first isolated by Dobrovolskaia-Zawadskaia and Kobozieff (1932) has been shown (Chesley and Dunn, 1936) to be due to interaction between the dominant gene for Brachyury T (Dobrovolskaia-Zawadskaia, *et al.*, 1934; Chesley 1935) and a recessive allele t^0. This strain, known as Line A, breeds true to taillessness because of a balanced lethal condition. The genetic composition of tailless animals of this line is Tt^0; the homozygotes TT die at 10–11 days after fertilization; t^0t^0 embryos die shortly after implantation (6th–7th day) and only the compounds Tt^0 survive. T and t^0 show no crossing-over and are probably alleles.

In a second tailless line,[1] also isolated by Dobrovolskaia-Zawadskaia and Kobozieff (1932) and designated as Line 29, a similar situation has now been found. Data in support of this statement are given in table 1. The strain breeds true to taillessness and produces small litters (Exp. 1). Crosses of tailless animals from this line by normal-tailed animals produced normal and short-tailed (Brachy) progeny (Exp. 2), indicating that Line-29 tailless is heterozygous for Brachyury (T). As in crosses of Line-A tailless, the ratios from Line-29 tailless females approximate 1 normal: 1 Brachy; those from tailless males indicate a marked excess of normals (Exp. 3). All F_1 normals when tested by Brachy ($T+$) transmitted taillessness,