BEHAVIOR OF NUCLEI AT CONJUGATION IN *PARAMECIUM AURELIA*. I. EFFECT OF INCOMPLETE CHROMOSOME SETS AND COMPETITION BETWEEN COMPLETE AND INCOMPLETE NUCLEI¹

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THE sexual processes of conjugation and autogamy in *Paramecium aurelia* involve synchronous division of nuclei, movement of nuclei into specific cytoplasmic regions, and survival of some nuclei and death of others. SONNEBORN (1951, 1955) has pointed out the importance of these phenomena for the problem of interactions between nucleus and cytoplasm and has especially emphasized the role of differentiated cytoplasmic regions. NANNEY (1953) has discussed similar problems in *Tetrahymena*. The present study describes the behavior of grossly deficient products of meiosis from haploid micronuclei and demonstrates that the genetic constitution of a nucleus can play an important and autonomous role in its behavior.

MATERIAL AND METHODS

Two stocks differing from each other in five independently assorting genes were used to study nuclear behavior, SONNEBORN's stock 90 of variety 1 and a synthetic stock called CD. CD is nearly isogenic with 90 except for the five genes *cl* (clear), *dp* (dumpy), *mt* (the one-type allele of the mating type gene), *d*⁶⁰ (the stock 60 allele of the gene for the D antigens), and *g*⁶⁰ (the stock 60 allele of the gene for the G antigens). Stock 90 contains the dominant wild type alleles of *cl*, *dp*, and *mt* and the stock-90 alleles of *d* and *g*. We are indebted to DR. G. H. BEALE for furnishing the stock 60 alleles of *d* and *g* in a stock 90 genetic background.

The genes *cl* and *dp* have been briefly reported by KIMBALL (1953). Clear animals lack the crystals found in the cytoplasm of wild type paramecia, and the character can be scored in individual paramecia even if they grow poorly. Dumpy animals differ from wild type in shape, and the character can only be scored in cultures which have grown at normal rate. The behavior of the *d* and *g* genes has been described by BEALE (1952, 1954). Any single paramecium may be immobilized by antisera against antigens controlled by one of these genes but not the other. In the present work, the D antigens determined by the *d* alleles were usually used and no attempt was made to test for the other gene. Animals heterozygous for *d*⁶⁰ and *d*⁹⁰ react with both anti-60D and anti-90D serum while the homozygotes react with only one. In most of the work only two genes of the five, *cl* and *d*, were used to follow nuclear behavior.

Haploid and haploid-diploid clones

Haploid clones were produced by irradiating CD with about 30 kr of X-rays and crossing to unirradiated 90. It will be reported in another paper that heavily irradiated animals frequently fail to contribute a male gamete nucleus to their unirradiated

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mates, and the nuclear apparatus in the latter frequently develops from a single haploid product of meiosis. Such haploids have the markers from stock 90 but none from CD. SONNEBORN *et al.* (1953) have briefly discussed the production of haploids by another technique and have pointed out that diploidization frequently occurs. In our experience, diploidization usually occurs in the early history of the clone if at all.

Two haploid clones, 3b and 19b, furnished the main body of data on haploids. The micronuclei of these clones had chromatin rings which were distinctly smaller than normal, and their haploidy was further confirmed by the death of all their progeny by autogamy. In the same experiment from which 3b was obtained, another clone, 20b, was found which, on microscopic examination, proved to contain one large and two small micronuclei. Occasionally more than two small nuclei were found but the nuclear constitution was, on the whole, quite stable. The breeding behavior of this clone is explicable on the assumption that the large nucleus was diploid and the two small ones were haploid. The clone had the phenotype expected if its macronucleus were derived from stock 90 only, and its breeding behavior shows that the diploid and at least one haploid micronucleus must have come from 90 only. It is unlikely, though not impossible, that the other haploid nucleus was from CD and not 90.

Crossing and determining the phenotype of the exconjugant clones

Pairs between the haploid or the haploid-diploid clones and stock CD animals were isolated. For convenience, they were left overnight and then the two members of the pair were put in separate containers using clear and dumpy to ascertain the cytoplasmic origin. The clones were allowed to multiply for 4 days in a small quantity of culture fluid and then classified for the amount of growth, the presence of crystals, and the serotype. Clones were classified as having crystals if some but not all the individuals contained them. Two categories of growth are distinguished in the paper, G and P. Clones classified as G reached a maximum population in 4 days; clones classified as P either died or failed to reach a maximum population in 4 days. In almost all instances, the two categories were markedly different from each other.

CONJUGATION

Normal

Conjugation in *Paramecium* has been described many times (e.g., SONNEBORN 1947; BEALE 1954), but a summary of the major nuclear events may be useful for an understanding of the rest of the paper. *P. aurelia* normally contains two micronuclei and a macronucleus. The micronuclei undergo meiosis nearly synchronously in the two conjugants to produce eight products in each. In each conjugant one of these enters the paroral cone, a cytoplasmic projection which presses into the side of the other conjugant. This product undergoes a mitotic division while the other seven products rapidly degenerate and are resorbed. One of the products of this mitosis, the male gamete nucleus, remains in the cone and eventually penetrates into the mate. The other, the female gamete nucleus, moves back into the body of the animal during the division at which it arises and later fuses with the male gamete

nucleus from the mate to form the synkaryon. A whole new nuclear apparatus, macronucleus and micronuclei, is derived from this synkaryon, following separation of the conjugants. The two exconjugant clones, one from each conjugant, thus come to have genetically identical nuclei since the two kinds of nuclei which fuse to form one synkaryon are identical with the two that fuse to form the other.

Two major aberrations of conjugation are relatively well known (e.g. SONNEBORN 1949; BEALE 1954). In cytogamy, the male gamete nucleus of each conjugant fuses with the female gamete nucleus of the same conjugant in a double autogamy. In macronuclear regeneration, the macronucleus of the exconjugant clone develops from a fragment of the old macronucleus instead of from the synkaryon. Thus the phenotype of the cytoplasmic parent is retained regardless of the origin of the synkaryon.

Cytological observations

All major stages in the conjugation of haploids with diploids have been observed cytologically though certain stages have been represented by only a few pairs. A number of pairs involving clone 20b were also examined including several in late meiotic and postmeiotic stages. No consistent differences have been found between haploid and diploid micronuclei in the rate at which they proceed through meiosis and the subsequent stages. The variations which have been found are no greater than are found in conjugation between normal diploids and do not seem to be in one direction. Likewise no differences have been found in the rate of macronuclear breakdown. The positions of the micronuclei in the cell at all stages up to and including the postmeiotic interphase seem to be quite variable both in pairs involving haploids and in pairs between normal diploids. The observations are in agreement with WICHTERMAN'S observations (1953) of extensive movement of the nuclei in living *P. caudatum* as a result of cytoplasmic cyclosis.

The only abnormality which was seen in 3b and 20b but not in normal diploids was the presence in several conjugants of 3, instead of the normal 2, gamete nuclei. The third gamete nucleus was located near the paroral cone but to one side of it while the other two were in the typical positions for male and female gamete nuclei. This observation suggests that occasionally two instead of one product of meiosis survive long enough so that they might take part in fertilization.

EXPERIMENTAL

Haploid $\times$ diploid crosses

The combined results of the crosses of 19b and 3b with diploid CD are shown in table 1. Two main facts can be seen by superficial examination. There are a large number of clones with poor growth, and there are a number of clones which appear to have obtained one of the markers from the haploid but not the other. This is the result expected if the single chromosome set in the haploid is segregated more or less at random to the meiotic products with the result that marker genes are lost and there is extensive aneuploidy in the hybrids.

However, this conclusion cannot be accepted without further consideration of the validity of phenotype determinations. For clones with good growth, the data in table 2

TABLE 1

Results of crosses between cl/cl d⁶⁰/d⁹⁰ diploids and cl⁺ d⁹⁰ haploids

Line	Clones from:				Clonal growth characteristics‡				
	Diploid conjugant		Haploid conjugant		Number of pairs of clones				
	Phenotype†		Phenotype						
	Crystals	Serotype	Crystals	Serotype	G/G	G/P	P/G	P/P	Total
1	+	60 and 90	+	60 and 90	0	0	0	7	7
2	+	60 and 90	—	60 and 90	0	0	0	2	2
3	+	60	+	60 and 90	0	0	0	4	4
4	+	60	+	60	0	0	0	3	3
5	—	60 and 90	+	60 and 90	0	1	0	4	5
6	—	60 and 90	—	60 and 90	0	0	0	2	2
7	+	60	+	90	0	0	0	1	1
8	—	60 and 90	+	90	0	0	0	1	1
9	+	60 and 90	?	?	0	0	0	1	1
10	—	60 and 90	?	?	0	0	0	6	6
11	+	60	+ or ?	?	0	1	0	1	2
12	+	?	+	60 and 90	0	0	0	7	7
13	+	?	+	90	0	0	0	2	2
14	+	?	+ or ?	?	0	1	0	18	19
15	—	60	+	60 and 90	2	8	0	1	11
16	—	60	+	60	0	4	0	0	4
17	—	60	—	60 and 90	0	1	0	0	1
18	—	60	—	60	1	2	0	1	4
19	—	60	+	90	2	7	3	0	12
20	—	60	+, —, or ?	?	0	28	0	7	35
21	— or ?	?	+	60 and 90	0	0	2	5	7
22	?	?	—	60 and 90	0	0	0	1	1
23	?	?	—	60	0	0	1	0	1
24	?	?	+	90	0	0	1	2	3
25	— or ?	?	+, —, or ?	?	0	0	0	32	32
Total.....									173

† +, crystals present; —, crystals absent. 60 means reacted with anti-60 serum; 90, reacted with anti-90 serum; 60 and 90, reacted with both sera. ? means that the phenotype could not be ascertained either because the clone died before 4 days or, rarely, because of an inconclusive observation.

‡ G means that the clone attained maximum population in 4 days; P, that it did not. The symbol to the left applies to the clone from the diploid conjugant; the symbol to the right, to the clone from the haploid conjugant.

show that the phenotype for crystals and serotype is an accurate indicator of the genotype. The table gives the results of a cross between stock CD and a diploid heterozygous for *cl* and *d*. The four main phenotypic classes occur in frequencies which do not differ significantly from the expected equal numbers, and the two members of the pair are alike except for the one aberrant pair. The regularity of the results demonstrates that the markers function satisfactorily in clones with good

TABLE 2
Cross of CD (cl/cl d⁶⁰/d⁶⁰) with a heterozygous (cl⁺/cl d⁶⁰/d⁹⁰) diploid

Phenotype of clones from:*				No. of pairs
CD conjugant		Heterozygous conjugant		
Crystals	Serotype	Crystals	Serotype	
+	60 and 90	+	60 and 90	49
+	60	+	60	43
—	60 and 90	—	60 and 90	53
—	60	—	60	34
—	60	+	60	1
Total				180

* Symbols are the same as in table 1. All clones grew well (G) except for the +60 clone of heterozygous cytoplasmic origin on the last line.

TABLE 3
Presence of the marker characters from the haploid conjugant in clones with marker characters from both parents (data from table 1)

Crystals*	Serotype*	Number of clones from	
		Diploid conjugant	Haploid conjugant
+	60 and 90	10	41
+	60	10	7
—	60 and 90	14	6

* Symbols as in table 1.

growth. However, the fact that the markers are satisfactory for clones with good growth does not demonstrate that they will be equally satisfactory for clones with poor growth. Evidence on this point is given in tables 3 and 4.

Table 3 summarizes the data from the clones in table 1 which were successfully scored for both crystals and serotype and seemed to possess markers from both parents. The *gain* of crystals and of the reaction with anti-90 serum by clones of CD cytoplasmic origin occurs in the frequency expected if there were random segregation of the two markers during meiosis in the haploid. Clones with both characters, with crystals only, and with the 90 specificity only occur in equal frequency. It would be expected that there would be about the same number of clones with neither marker, and in lines 15 through 20 of table 1 there are 12 clones with poor growth which have neither. The clones with good growth in these lines represent a separate problem to be considered presently. The close fit to expectation makes it probable that gain of these characteristics is complete or nearly so even in clones with quite poor growth.

A further check on this conclusion is provided by the data in table 4. The table shows the phenotypes of clones of CD cytoplasmic origin from a cross of a haploid derived from CD with a diploid of stock 90, the reverse of the crosses in tables 1 and 3. Of the 29 poorly growing clones which showed at least one marker from 90, only 4 failed to show the other. Three other clones showed neither marker but these may

TABLE 4

The phenotypes of clones of haploid cytoplasmic origin from a cross of cl d⁶⁰ haploids with cl⁺/cl⁺ d⁹⁰/d⁹⁰ diploids

Phenotype*			Number of Clones
Crystals	Serotype	Growth	
+	60 and 90	P	21
+	60	P	2
+	90	P	4
—	60 and 90	P	2
—	60	P	3
+	60 and 90	G	2
+	90	G	1
Dead within 4 days or incompletely classified.....			55
Total.....			90

* Symbols are the same as in table 1.

have obtained no nucleus at all from 90. In this cross, any clone which obtained a nucleus from 90 should show both markers unless phenotypic lag prevented it. Thus it can be calculated that between 84 and 93 percent of the clones show the phenotype corresponding to their genotype in respect to the markers gained from the 90 parent.

The situation is much less favorable for detecting loss of the markers. The second column in table 3 shows that most of the clones from the haploid parent have the phenotype determined by both cl^+ and d^{90} even though it would be expected that half would lose one or the other. Similar conclusions can be drawn for d^{60} from table 4. On the assumption of random loss of markers from about half the clones, it can be calculated from tables 3 and 4 that loss of markers will be detected in only about a quarter of the cases in which it occurs. This conclusion is based on the assumption that marker genes are equally likely to be missing from the male and female gamete nuclei of the haploid. Other explanations of the data cannot be completely excluded. For example, the extra gamete nuclei, which were observed cytologically, may, by multiple fusion, make loss of markers less likely in the female contribution to the synkaryon. However, the phenotypic lag explanation seems quite probable and will be accepted in the rest of the discussion.

One other possible difficulty with the markers can be eliminated. A considerable number of poorly growing clones have been obtained both by autogamy in haploids of CD origin and by autogamy in irradiated clones of CD. In no case have these poor clones developed crystals. Thus the conditions causing poor growth are not sufficient to cause crystals to be formed in animals which lacked them before fertilization. The cl^+ allele must be introduced.

With this information on the markers, it is possible to draw conclusions on certain aspects of nuclear behavior from the data in table 1. The clones of diploid cytoplasmic origin in lines 1 through 14 of the table are all of the phenotype expected of hybrids

between the two parents. The lag phenomenon makes it impossible to establish beyond doubt that the clones received a nucleus from the diploid parent, although this seems quite probable. However, it is established that they obtained a male gamete nucleus from the haploid and that this nucleus frequently lacked one or the other of the marker genes. In most cases, the clones grew poorly as would be expected if they were extensively aneuploid; but in three cases there was good growth, at least in the first 4 days. Two of these latter clones lacked a marker gene and so were probably aneuploid for at least one chromosome. Random segregation of the approximately 40 pairs (DIPPEL 1955) of chromosomes would not be expected to give rise this frequently to clones with only one chromosome missing. Non-random segregation, selection of more complete meiotic products, resistance to the deleterious effects of aneuploidy, the presence of inert chromosomes or probably some combination of these is required. All that can be said at this time is that the chromosomes bearing *cl* and *d* appear to segregate at random and nuclei lacking them do not suffer a marked selective disadvantage compared to nuclei containing them but lacking other chromosomes. Thus the information on the male gamete nucleus from the haploid is in general agreement with a more or less random distribution of the single chromosome set to the meiotic products, but certain departures from this simple situation probably occur.

The G clones in lines 15 through 20 of the table show that the haploid often fails to contribute any detectable nucleus to the diploid mate. These clones grew well and, in addition to lacking crystals and reacting only with anti-60 serum, were dumpy and mating type I as would be expected if they had obtained nuclei only from CD. If it is assumed that all clones of diploid origin which grew poorly received a nucleus from the haploid, then the clones which did not receive such a nucleus make up about $\frac{1}{3}$ of all pairs. It can be seen from table 1 that many of these pairs were true conjugants since the d^{60} allele was contributed to the haploid mate. Thus there is good evidence that failure of the male gamete nucleus of the haploid is a specific aberration of the conjugation process. However some pairs, especially those in line 19, may have been cytogamous or non-conjugants.

The table also shows that the diploid contributed a male gamete nucleus to the haploid in a large fraction of the cases in which sufficient evidence is available. A reaction with anti-60 serum was found in 59 of the 78 clones for which the serotype was determined. Actually the 6 G clones of haploid origin in lines 19 and 24 were probably macronuclear regenerates or non-conjugants and so should not be included in the critical cases. Moreover on the basis of the data for d^{90} in table 4, about 10 percent of the clones which obtained d^{60} should fail to show it because of lag. When these corrections are made, it can be calculated that in about 90% of all pairs in which a test was possible, the diploid contributed a male gamete nucleus to the haploid.

It can also be shown that the female gamete nucleus from the haploid member functions much more frequently than the male. This cannot be demonstrated directly by the marker genes because phenotypic lag introduces too much uncertainty. However, most of the clones which were of haploid cytoplasmic origin, grew poorly, and reacted with anti-60 serum were almost surely hybrids. The reaction with

TABLE 5

Comparison of the results of conjugation between diploids and diploids, haploids, or haploid-diploids with and without about 5 kr of X-rays

Classification of pair for growth and crystals*	Number of pairs as percentage of total number						
	CD $\times$ 90 diploid $\times$ diploid		CD $\times$ 3b or 19b diploid $\times$ haploid		CD $\times$ 20b diploid $\times$ haploid-diploid		
	0 kr	5 kr†	0 kr	5 kr‡	0 kr	5 kr‡	E†
G+/G+	93.7	85.9	0.0	0.0	90.4	27.1	28.6
G-/any	2.2	9.6	31.8	28.4	6.2	32.9	22.2
Other	4.1	4.4	68.2	71.6	3.4	40.0	49.2
Total number of pairs	270	270	173	88	177	85	

* (Symbols as in table 1). The clone from the CD (diploid) member is shown to the right; the clone from other member, to the left.

† Distribution expected if one third of the pairs had the distribution of the diploid $\times$ 5 kr diploid group and two thirds had the distribution of the diploid $\times$ 5 kr haploid group.

‡ In each case the CD member was not irradiated and the other member was irradiated at least one day prior to conjugation.

anti-60 serum shows that they obtained a gamete nucleus from the diploid and if this were the only source of their nuclei they should have been simple haploids which normally grow well. The poor growth is evidence for aneuploidy resulting from a nuclear contribution from the haploid parent.

Haploid-diploid $\times$ diploid crosses

The mixed haploid-diploid clone 20b was crossed with CD, and the results are tabulated in table 5. Crosses between two diploids and between diploids and haploids are shown for comparison. The cross with unirradiated 20b gave the same results as the cross between two diploids. However, the cytological observation that one diploid and two haploid nuclei were present is confirmed by the cross of irradiated 20b with unirradiated CD. The results are very nearly those expected if one third of the pairs were like those from the cross of the unirradiated with the irradiated diploid and two thirds like those from the cross of the unirradiated diploid with the irradiated haploid. Thus the diploid nucleus of unirradiated 20b functions almost to the exclusion of the haploid nuclei, but this selective advantage is essentially eliminated by 5 kr of X-rays. This finding will be discussed further in the second paper of this series and is introduced here only to demonstrate that the nuclear constitution of 20b is the one assigned it by microscopic examination. This is also supported by the observation that about half of the poorly growing clones from the CD member of the cross with irradiated 20b had crystals, and half did not as would be expected if they were aneuploids derived from meiotic products of the haploid nuclei.

DISCUSSION

There are at least the following steps between meiosis and synkaryon formation: (1) entrance of a meiotic product into the paroral cone, (2) disintegration of the

meiotic products outside the cone, (3) division of the cone nucleus to form the gamete nuclei, (4) exchange of male gamete nuclei and (5) fusion of male and female gamete nuclei. SONNEBORN (1955) has shown that one factor in the first step is the expansion of the macronucleus as it breaks up into a skein. A clone (d59) in which this breakdown occurred less rapidly than usual failed to get any meiotic products into the cone. This evidence would be consistent with the view that a nucleus was carried more or less passively into the cone. However, the very fact that conjugation proceeds normally in the overwhelming majority of pairs between normal clones suggests a more active attraction into the paroral cone; otherwise nuclei should frequently fail to enter. This is probably implied by SONNEBORN's use (1955) of the phrase "sphere of influence" of the paroral cone.

The crosses with 20b show that the nature of the individual micronuclei or their products is also of importance. The cytological observations show no clear difference between the haploid and diploid nuclei in the rate at which they pass through the earlier stages of meiosis. The observations of normal diploids show that there is sufficient variation in position and timing so that differences which were not readily detected at these stages would be expected to be without any appreciable effect. They would be lost in the general random variations of the process. Thus the difference in behavior probably occurs during the very last stages of meiosis or in the postmeiotic period.

One explanation which, at first thought, is appealing is that chromosome bridges formed by non-homologous crossing over in the haploid are involved. However, this seems most improbable. If such bridges were able to nearly exclude the haploid nuclei from participation in fertilization, they should appreciably delay both meiotic divisions in almost all haploids. No evidence for this was found. Moreover, cytological observations of normal diploids suggests that there is an interphase following the second meiotic division during which considerable movement of the meiotic products occurs. Thus even if bridges slightly delayed this division, the delay would probably have little effect on the position of the nuclei at the time of entrance into the cone. In fact, if anything, it should bring the delayed nuclei nearer to the cone not further away since products of the first meiosis often start their second division in the general vicinity of the cone. Finally, it seems unlikely that chromosome bridges would have any appreciable effect on the separation of daughter nuclei because the spindles are very large compared to the chromosomes and most of the separation of daughter nuclei appears to involve an extensive elongation and attenuation of the central part of the spindle.

Thus it seems very probable that the failure of the haploid nuclei to contribute to fertilization in the cross with unirradiated 20b is to be attributed to the genetic deficiency of their meiotic products and not to the original haploidy or to mechanical difficulties imposed by chromosome bridges.

The second step, death of nuclei outside the cone, has been attributed by SONNEBORN (1955), on the basis of the behavior of d59, to a lethal effect of the cytoplasm on nuclei lying outside the cone. The cytological evidence from the present work for survival of meiotic products outside the cone suggests that the region in which nuclei can be located and survive is probably somewhat larger than the cone itself.

In normal conjugation, only one nucleus remains near the cone; but in haploid conjugation, more than one may stay near it, presumably because of defective nuclear behavior. This hypothesis agrees with the cytological observation that extra nuclei, when found, are near the cone, and it does not require the improbable assumption that the genetically deficient nuclei are less likely to be killed than normal ones.

It has been shown that the haploid frequently fails to contribute a male gamete nucleus to its diploid mate, although it may contribute a functional female nucleus to itself. A number of explanations of this observation can be suggested, and several examples will be given here: (1) no nucleus reaches the cone, but at least one outside the cone survives and functions as a female nucleus; (2) a deficient cone nucleus fails to divide and remains behind as a female nucleus; (3) the cone nucleus fails to form a functional gamete nucleus of either kind, and the female nucleus comes from a nucleus surviving outside the cone; and, (4) the cone nucleus forms both male and female nuclei, but the former fails to reach the female nucleus of the normal mate. The evidence for competition for entrance into the cone provided by the cross with 20b makes (1) the least probable since, with many nuclei available, it is quite unlikely that all could fail to reach the cone. The other explanations do not involve this difficulty, and attribute the failure of the male gamete nucleus to defective behavior of the cone nucleus itself. The differential failure of the male gamete nucleus in these various schemes is attributed either to a greater complexity of the activity of the male nucleus making it more likely to fail than the female, or to a greater number of nuclei which can serve as a source of the female nucleus so that when one source, the nucleus in the cone, fails other sources are often available.

Whatever the precise nature of the failure, the same arguments which were used in connection with entrance of a meiotic product into the cone can be used to support the view that it is the genetic constitution of the meiotic products themselves which interferes with the formation of a functional male nucleus. However, it is necessary first to exclude the possibility that there is some effect of haploidy on the whole animal which then interferes with the formation of the gamete nucleus. In the first place, cytological evidence suggests that the macronucleus is the same size in haploids and diploids. In other words, the degree of polyploidy reached is not dependent on the number of chromosomes in the nucleus which gives rise to the macronucleus. The major characteristics of the whole animal are determined by the macronucleus and so it is unlikely that haploidy of only the micronuclei would have any general effect. In the second place, the results of irradiation of 20b are very difficult to understand on this basis. The fraction of pairs in which the male gamete nucleus from irradiated 20b failed to function is not significantly different from that expected if the haploid nucleus contributed gamete nuclei $\frac{2}{3}$ of the time. If a systemic effect of the radiation were involved, it would have to be assumed that somehow it duplicated rather precisely the results expected if only the nuclear constitution of the meiotic products was important. This possibility seems very remote, especially since the same dose of radiation had practically no effect on the formation of male nuclei by ordinary diploids and haploids (see table 5).

On the basis of the preceding argument, it can be concluded that the behavior

of nuclei involves localized interactions of the nucleus' own genome with the immediately surrounding cytoplasmic environment. The latter may, of course, be under the control of other nuclei within the common cytoplasmic mass. The deficient nature of the meiotic products of haploid micronuclei cannot be adequately supplemented by products diffusing to them from other nuclei in the same cytoplasm and so their behavior is, in this respect, autonomous. This situation contrasts with the more usual finding with heterokaryons that genetically diverse nuclei in a common cytoplasm complement each other and act as though the diverse genomes were contained in a common nucleus. However, there is some evidence in heterokaryons of fungi that the distribution of the parts of the total genome to the various nuclei may be of importance though the cases are not entirely similar. PONTECORVO (1952) discusses this and other problems concerning the localization of nuclear processes within the cell.

SUMMARY

1. Crosses were made between a diploid marker stock of *Paramecium aurelia* and clones having haploid or both haploid and diploid micronuclei and conclusions were drawn from the genetic and some cytological data about the behavior of the meiotic products of the haploid.

2. In conjugation between haploids and diploids, the diploid usually contributes a male gamete nucleus to the haploid, but the haploid frequently fails to contribute a male nucleus to the diploid. However, even in this latter case, the haploid generally contributes a female gamete nucleus to itself.

3. The gamete nuclei of the haploid are usually grossly deficient as a result of more or less random segregation of the single chromosome set at meiosis.

4. When both haploid and diploid micronuclei are present in a common cytoplasm, the gamete nuclei almost always come from the diploid. This selective advantage can be almost completely eliminated by 5 kr of X-rays.

5. The two major abnormalities in the nuclear behavior of the meiotic products of haploid micronuclei are, therefore, (1) a frequent failure to form a functional male gamete nucleus and (2) an inability to compete successfully with products of diploid micronuclei for the formation of gamete nuclei.

6. Reasons are given for believing that these abnormalities are the result of the deficient genome of the meiotic products and that nuclear behavior is controlled by interactions between the nucleus' own genome and the surrounding cytoplasm.

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