Genomic Exclusion in *Tetrahymena:* Genetic Basis*

SALLY LYMAN ALLEN

Zoology Department, University of Michigan, Ann Arbor, Michigan

SYNOPSIS. Genomic exclusion is an aberration that occurs during conjugation in variety 1 of *Tetrahymena pyriformis.* Instead of containing markers from both parents, the outcross pairs are either homozygous for all the genes of one parent (unilateral genomic exclusion); or, some of the pairs are homozygous for the genes of one parent and other pairs are homozygous for the genes of the other parent (bilateral genomic exclusion). This phenomenon was first demonstrated in the C strain: some stocks evoke unilateral genomic exclusion; others, bilateral genomic exclusion. C*, inbred for 5 generations, was used to explore this phenomenon in some detail since unilateral genomic exclusion of C genes occurs in almost all pairs in outcrosses of C*. In a mating of C*, both exconjugants are recovered, both are diploid and similar in phenotype. Using morphological markers, C* can be shown to participate in the mating; therefore, C* does not induce illegitimate matings of the normal mate. When the normal mate is heterozygous for alleles (H^A/H^D) not present in C*, 3 classes of offspring $(H^A/H^A, H^A/H^D$ and $H^D/H^D)$ are produced in a 1:2:1 ratio. These observations indicate that 2 meiotic products of the normal mate unite to form the syncarya. The genetic ratios obtained in 1 and 2 factor crosses limit the possible cytogenetic bases for genomic exclusion. They suggest that 1 of the 4 haploid nuclei replicates and the replica fuses randomly with any 1 of the 4 nuclei. The 2 schemes of nuclear behavior (single fertilization, double fertilization) that would satisfy these requirements have not yet been resolved.

FOUR years ago an unexpected observation was made in variety 1 of *Tetrahymena pyriformis.* In an outcross of a member of the inbred C strain to the B strain, most of the progeny behaved as if they were B/B homozygotes rather than heterozygotes. This peculiar pattern of segregation was initially observed in crosses involving a new marker, *E-1,* and it was confused at first with properties ascribed to *E-1* (1). Later, abnormal segregation was also observed at other loci: at *mt*(2), *E-2, H* and *P-1.* Since all genes seem to be involved in this phenomenon, the nuclei derived from the C parent appeared not to participate in conjugation. The term, genomic exclusion, will be adopted for this phenomenon, since it seems to be an apt descriptive title.

The frequency of pairs manifesting genomic exclusion varies when different members of the C strain are used as the C parent. Some C stocks behave completely normally in crosses. Some give rise to only a few pairs that have resulted from genomic exclusion. Others give rise to only a few normal pairs and the majority of pairs manifest genomic exclusion. C*, inbred 5 generations (C-5573), is an example of the latter type. In outcrosses of C* almost all pairs show genomic exclusion. In this report the results of crosses of C* will be described in some detail. The observations establish the genetic consequences of genomic exclusion and permit extrapolation to specific sequences of nuclear behavior.

MATERIALS AND METHODS

Inbred strains. Inbred strains A, B and C will be specifically mentioned in this report, although outcrosses of C* to strain D also resulted in genomic exclusion. Strains A and B

were derived from a cross of WH-6 and WH-14; strain C was probably derived from a cross of UM-226 and strain B (see 1, 9). Most of the inbred strains are now in the 12th or 13th generation of inbreeding. The genotypes of strains A, B and C are the following:

A	B	C	
mt^A/mt^A	mt^B/mt^B	mt^C/mt^C	(9)
H^A/H^A	H^D/H^D	H^E/H^E	(12)
$E\text{-}1^B/E\text{-}1^B$	$E\text{-}1^B/E\text{-}1^B$	$E\text{-}1^C/E\text{-}1^C$	(3)
$E\text{-}2^B/E\text{-}2^B$	$E\text{-}2^B/E\text{-}2^B$	$E\text{-}2^C/E\text{-}2^C$	(3)
$P\text{-}1^A/P\text{-}1^A$	$P\text{-}1^B/P\text{-}1^B$	$P\text{-}1^B/P\text{-}1^B$	(5)

Methods. Tetrahymena were grown on either bacterized medium (Cerophyl rye grass inoculated with *Aerobacter aerogenes*) or axenic medium (1% proteose-peptone). Crosses of cells grown in both media have been made, with similar genetic results(1,3). More recently, we have returned to making all our crosses with cells grown on bacterized medium, since we have devised a method to prevent selection of sublines when transferring a sample to the peptone medium for enzyme analysis(5).

Mating type and serotype tests were performed on cells grown in bacterized medium(8,11). For analysis of enzymes, extracts of peptone grown cultures were employed(1,3,4,5).

The various forms of a given enzyme are revealed by starch-gel electrophoresis. *E-1* and *E-2* control alternative forms of 2 different esterases(3); *P-1* controls alternative forms of an acid phosphatase(5).

RESULTS

Properties of Genomic Exclusion

Genomic exclusion is demonstrated for 3 of the loci in Table 1. *1, 2* and *3* are crosses of normal stocks. Crosses *1* and *2* give rise to an array of progeny typical for each type of homozygote. Cross *3* gives rise to an array of progeny typical for each type of heterozygote. *4* and *5* are crosses of C*. Crosses *2* and *4* result in distributions that are comparable. But, in

TABLE 1. *Genomic exclusion in outcrosses of C*.*

a. Segregation of alleles at E-1

	Cross	No. of pairs with phenotype: E-1B	E-1BC	E-1C	Total No. pairs
1.	E-$1^B/E$-$1^B \times E$-$1^B/E$-1^B	35	0	0	35
2.	E-$1^C/E$-$1^C \times E$-$1^C/E$-1^C	0	0	30	30
3.	E-$1^B/E$-$1^B \times E$-$1^C/E$-1^C	0	10	0	10
4.	E-$1^C/E$-$1^C \times C^*$	0	0	22	22
5.	E-$1^B/E$-$1^B \times C^*$	25	1	0	26

b. Segregation of alleles at mt (at 30°C)

	Cross	Frequency of mating types: I	II	III	IV	V	VI	VII	Total No. caryonides
1.	$mt^B/mt^B \times mt^B/mt^B$	0	17.8	2.2	62.5	3.4	7.7	6.4	594
2.	$mt^C/mt^C \times mt^C/mt^C$	44.8	19.8	1.7	0	0.9	32.8	0	116
3.	$mt^B/mt^B \times mt^C/mt^C$	25.8	13.3	0	24.2	3.3	29.2	4.2	120
4.	$mt^C/mt^C \times C^*$	48.4	23.3	3.3	0	1.7	23.3	0	60
5.	$mt^B/mt^B \times C^*$	0†	17.0	1.5	71.0	3.5	4.0	3.0	200

c. Segregation of alleles at H

	Cross	No. of pairs with phenotype: Ha	Hae	He	Total No. pairs
1.	$H^A/H^A \times H^A/H^A$	62	0	0	62
2.	$H^E/H^E \times H^E/H^E$	0	0	73	73
3.	$H^A/H^A \times H^E/H^E$	0	44	0	44
4.	$H^E/H^E \times C^*$	0	0	31	31
5.	$H^A/H^A \times C^*$	84	2	0	86

† From one exconjugant clone that was a selfer, subclones were isolated that were mating type I.

the outcrosses, differences in segregation are observed: cross *3* results in the expected observation, while cross *5* shows distorted segregation. Thus, in cross *a3* E-1BC pairs were observed, while in cross *a5* most of the pairs were E-1B, and only 1/26 pairs showed the expected phenotype. In cross *c3* Hae pairs were observed, while in cross *c5* most of the pairs were Ha, and only 2/86 of the pairs showed the expected phenotype. The mating types are also similarly distorted in their segregation. The examples given in Table 1*b* were obtained from crosses made at 30°C. The same pattern was found in crosses carried out at 23°C. All 7 mating types are produced if a cell is heterozygous for mt^B/mt^C (9); moreover they are produced in certain characteristic frequencies depending upon temperature. Cross *3* resulted in an array of types typical for a heterozygote. However, cross *5* resulted in an array of types characteristic of mt^B/mt^B. In only 1/112 pairs could mating type I be ex-

tracted. This pair was the same pair that showed the phenotype E-1BC, expected of a heterozygote.

A number of different crosses have been made in which the exconjugants were separated. Both exconjugants give rise to viable cultures. When these are tested for a number of different markers (*E-1*, *E-2*, *H* and *P-1*), the phenotypes of the 2 exconjugants from the same pair were found to be alike. Table 2 shows that in very few pairs (7/97) did only 1 of the exconjugants of a pair die. It also shows that genomic exclusion affects both exconjugants of a pair. In 62 of the pairs in which both exconjugants were recovered, both were Ha. One normal pair was produced. Both exconjugants were He in phenotype but, when testcrossed, the genotype was H^A/H^E (H^E tends to be preferentially expressed in the heterozygote; see Nanney *et al.*, 13).

Genomic exclusion does not result from mere changes in expression of the alleles in a heterozygote.

TABLE 2. *Genomic exclusion in serotypes of F1 exconjugants.*

Cross	Viability of exconjugants No. pairs isolated	Both dead	One dead	Both alive	No. pairs in which both exconjugants were: Ha	Hae	He	No. pairs in which viable exconjugant was: Ha	Hae	He
A (H^A/H^A) × C*Small (H^E/H^E)	45	25	5	15	14	0	1†	5	0	0
A (H^A/H^A) × C*GIANT (H^E/H^E)	52	2	2	48	48*	0	0	2	0	0

* Testcrosses of 7 pairs showed them to be H^A/H^A.   † Testcross showed that this pair was H^A/H^E.

TABLE 3. *Testcrosses of pairs produced by genomic exclusion.*

| Cross | No. pairs | Frequency of mating types at 30°C: | | | | | | | |
		I	II	III	IV	V	VI	VII	Total
F1 × F1	4	0	18.2	1.5	62.8	5.3	6.6	5.7	457
F1 × C*	5	0	15.8	3.5	61.5	3.7	9.3	6.2	517
F2 × F2	5	0	20.4	2.4	58.6	3.0	8.0	7.7	338
mt^B/mt^B × mt^B/mt^B		0	17.8	2.2	62.5	3.4	7.7	6.4	594

Abnormal pairs are genetically homozygous for the alleles of the normal parent. Testcrosses of 5 F1 pairs and 5 F2 pairs showed that their mating type genotype was mt^B/mt^B (Table 3). Several of these pairs were also tested for their *E-1* genotype; all were $E-1^B/E-1^B$. The viability of these crosses was good, and it was not less than the viability of crosses of normal pairs. Crosses of haploid clones are highly inviable(6). Therefore, the abnormal pairs resulting from genomic exclusion are not only homozygous but they are also diploid.

Testcrosses of some pairs from an outcross of C* showed that they are genetically heterozygous. For example, 20 F2's descended from the E-1BC pair described in Table 1 (cross a5) were tested for their *mt* and *E-1* genotype. Thirteen were mt^B/mt^C, 6 were mt^B/mt^B, and 1 was mt^C/mt^C. Eleven were $E-1^B/E-1^C$, 2 were $E-1^B/E-1^B$ and 7 were $E-1^C/E-1^C$. Since some pairs produced in an outcross of C* can be shown to be genuine heterozygotes, segregation apparently is, in rare exceptions, normal.

The most informative observation was made when hybrids (B/C) were crossed to C*. Three classes of pairs were formed: B homozygotes, heterozygotes, and C homozygotes. The genotypes of these classes were confirmed by testcrosses. These classes appeared in frequencies suggestive of a 1:2:1 ratio. This type of cross is demonstrated for *E-1* in Table 4 for C* as well as C' (C-6586), a derivative of C*. A total of 16 pairs were E-1B, 37 were E-1BC and 21 were E-1C. This distribution fits a 1:2:1 ratio very closely (p = .8).

A very simple interpretation of this observation (14) is that genomic exclusion arises as a consequence of induced selfing of normal cells by C*. Induced selfing has been observed by L. L. Larison and R. W. Siegel in *Paramecium bursaria*(7). According to this interpretation, the abnormal pairs would arise as a result of illegitimate matings induced by the presence of C*. Genuine matings between C* and a normal cell would give rise to normal pairs. This interpretation accounts for all of the observations. Thus, in a mating of C* and a B/C heterozygote a 1:2:1 ratio of B/B, B/C and C/C pairs would be expected.

In order to test this hypothesis, it was necessary to find out whether the mates of a pair formed in a mating of C* by a normal cell were both normal cells or whether one was a normal cell and the other was C*. This problem was solved by experiments in which the C* cell was marked.

Two lines of evidence show that C* does not induce selfing of normal cells but that C* *is* one of the mates. Ten to 20 pairs were isolated into separate depressions and serotype tests performed (Table 5).

TABLE 5. *Identification of pairs formed after various matings by specific antisera.*

| Mating | H genotypes | Response of pairs to antiserum against: | | |
		Ha	Hd	He
A × A	H^A/H^A × H^A/H^A	+	—	—
B × B	H^D/H^D × H^D/H^D	—	+	—
C × C	H^E/H^E × H^E/H^E	—	—	+
A × C	H^A/H^A × H^E/H^E	+	—	+
B × C	H^D/H^D × H^E/H^E	—	+	+
A × C*	H^A/H^A × H^E/H^E	+	—	+
B × C*	H^D/H^D × H^E/H^E	—	+	+
C × C*	H^E/H^E × H^E/H^E	—	—	+

As controls, tests were made on pairs obtained from matings within inbred strains (A × A; B × B; C × C) and in matings between a normal C and A or B (A × C; B × C). These tests were then compared to matings of C* and A, B or C (A × C*; B × C*; C × C*). In the inbred series and in the mating of C × C* immobilization of pairs occurred *only* with homologous antiserum; whereas, in the outcrosses (A × C; B × C) immobilization of pairs occurred with *both* parental antisera. Significantly, immobilization occurred with anti-He in the outcrosses of C* (A × C*; B × C*).

A second line of evidence involved a morphological marker for C*. A subline of C* pure for GIANTS was used in a mating to normal-sized cells of strains A or B. The pairs contained one GIANT and one small member. The size difference gradually dimin-

TABLE 4. *Segregation of E-1 alleles in progeny of F1 (B × C) crossed to C* or C'.*

| Cross | No. pairs with phenotype: | | | |
	E-1B	E-1BC	E-1C	Total
F1 (B × C*) × C*	10	15	9	34
F1 (B × C') × C'	6	22	12	40
Total observed	16	37	21	74
Expected 1:2:1	18.5	37	18.5	p = .8

ished with time. However, when GIANT-small pairs were isolated and checked at two-hour intervals until separation, the size difference was usually still noticeable at the time the exconjugants separated. This was a viable mating, and genomic exclusion was observed in the separately tested exconjugant cultures (Table 2).

Genomic exclusion must, therefore, involve a mechanism other than induced selfing. Somehow more than one meiotic product of the normal cell must participate in reconstituting the diploid nucleus to account for the unexpected classes of pairs.

Genetic Hypotheses for Genomic Exclusion

Several cytogenetic schemes can be envisioned whereby 2 meiotic products contributed from the normal mate unite to form the syncarya. The various schemes, however, fall into 3 categories depending upon (a) the number of haploid nuclei involved and (b) the types of unions made in forming the diploid nucleus. The probabilities of formation of the various diploid nuclear types differ for each of the 3 hypotheses. These generate different sets of genetic expectations for 1 and 2 factor crosses.

In discussing these schemes some explanation of the notation used may be helpful. At a hypothetical locus, A, are alleles A and a. After MI and MII 2 haploid nuclei will contain A, A^1 and A^2, and 2 haploid nuclei will contain a, a^1 and a^2. The superscripts refer to the fact that the residual genetic background will differ for each nuclear type. Union of A^1 with A^1, or A^2 with A^2, or a^1 with a^1, or a^2 with a^2, to form the syncaryon will give rise to diploid cells that are completely homozygous for all their genes, although each of the 4 types of diploid cells will differ in their combination of genes. Union of A^1 with A^2, or a^1 with a^2, will result in diploid cells that are homozygous for A or for a, but these cells will not be homozygous for all other genes. Each of the 3 hypotheses to be considered gives rise to different probabilities of formation of these various diploid nuclear types.

Hypothesis 1: Suppose that reunion of the haploid nuclei takes place after the 2nd meiotic division. At this stage there are 4 haploid nuclei. If any 2 of these 4 haploid nuclei fuse, this gives rise to 6 possible types of diploid nuclei. A detailed analysis of this scheme follows:

Haploid nuclei:	Possible types of diploid nuclei resulting from random fusion of 2 of 4 haploid nuclei
A^1, A^2, a^1, a^2	A^1A^2, A^1a^1, A^1a^2
	A^2a^1, A^2a^2
	a^1a^2

If these possible types of diploid nuclei are classified according to their A genotype, *i.e.*, AA, Aa or aa, a 1:4:1 distribution would be expected:

Distribution of nuclear types among pairs classified as:

AA	Aa	aa
A^1A^2	A^1a^1	a^1a^2
	A^1a^2	
	A^2a^1	
	A^2a^2	

If AA or aa pairs are selected and examined for segregation of alleles at a 2nd locus, B, a "classical" F2 type of distribution of pairs should be observed: 1 BB: 2 Bb: 1 bb. Distortion away from this type of distribution would *not* be expected since diploidy is *always* reconstituted by fusion of *non-identical* haploid nuclei (A^1A^2 or a^1a^2).

Hypothesis 2: Suppose that reunion of the haploid nuclei occurred after the 3rd meiotic division. At this stage there are 5 haploid nuclei, 2 of which are replicas. If random fusion of any 2 of the 5 haploid nuclei took place, this would give rise to 4 sets of 10 possible types of diploid nuclei. For clarification, this scheme is detailed below:

Haploid nuclei:	Possible types of diploid nuclei resulting from random fusion of 2 of 5 haploid nuclei:
A^1, A^1, A^2, a^1, a^2	A^1A^1, A^1A^2, A^1a^1, A^1a^2
	A^1A^2, A^1a^1, A^1a^2
	A^2a^1, A^2a^2
	a^1a^2
A^2, A^2, A^1, a^1, a^2	A^2A^2, A^1A^2, A^2a^1, A^2a^2
	A^1A^2, A^2a^1, A^2a^2
	A^1a^1, A^1a^2
	a^1a^2
a^1, a^1, A^1, A^2, a^2	a^1a^1, A^1a^1, A^2a^1, a^1a^2
	A^1a^1, A^2a^1, a^1a^2
	A^1A^2, A^1a^2
	A^2a^2
a^2, a^2, A^1, A^2, a^1	a^2a^2, A^1a^2, A^2a^2, a^1a^2
	A^1a^2, A^2a^2, a^1a^2
	A^1A^2, A^1a^1
	A^2a^1

When these nuclear types are classified according to their A genotype, a 1 AA: 3 Aa: 1 aa distribution of pairs would be expected:

Distribution of nuclear types among pairs classified as:

AA	Aa	aa
1 A^1A^1	6 A^1a^1	1 a^1a^1
1 A^2A^2	6 A^1a^2	1 a^2a^2
6 A^1A^2	6 A^2a^1	6 a^1a^2
	6 A^2a^2	

Notice that $\frac{1}{4}$ of the AA pairs and $\frac{1}{4}$ of the aa pairs are formed by the reunion of identical nuclei (A^1A^1, A^2A^2, or a^1a^1, a^2a^2). Cells possessing such nuclei should, therefore, be "pseudoautogamous," or homozygous for *all* their genes. If pairs homozygous for A or for a are selected and screened for their B genotype, instead of a 1 BB:2 Bb:1 bb ratio of pairs, a predictable distortion towards homozygosis can be computed. Unless both loci are closely linked to their centromeres, a 5:6:5 ratio of pairs would be expected for B. This is demonstrated for the nuclear types A^1A^1 and A^1A^2:

	BB	Bb	bb
.25 A¹A¹	.125	0	.125
.75 A¹A²	.1875	.375	.1875
	.3125	.375	.3125

Hypothesis 3: Suppose that reunion of the haploid nuclei takes places after the 3rd meiotic division when there are 5 haploid nuclei, 2 of which are replicas. But, instead of random fusion of any 2 haploid nuclei, the replica fuses with any 1 of 4 haploid nuclei. In this case, 4 sets of 4 possible types of diploid nuclei would be expected, as depicted below:

Haploid nuclei:	Possible types of diploid nuclei resulting from fusion of replica with any 1 of 4 haploid nuclei:
A¹, A¹, A², a¹, a²	A¹A¹, A¹A², A¹a¹, A¹a²
A², A¹, A², a¹, a²	A¹A², A²A², A²a¹, A²a²
a¹, A¹, A², a¹, a²	A¹a¹, A²a¹, a¹a¹, a¹a²
a², A¹, A², a¹, a²	A¹a², A²a², a¹a², a²a²

Classification of the nuclear types according to their *A* genotype leads to a 1 *AA*:2 *Aa*:1 *aa* distribution of pairs:

Distribution of nuclear types among pairs classified as:

AA	Aa	aa
1 A¹A¹	2 A¹a¹	1 a¹a¹
1 A²A²	2 A¹a²	1 a²a²
2 A¹A²	2 A²a¹	2 a¹a²
	2 A²a²	

In this case ½ of the *AA* pairs and ½ of the *aa* pairs involve the reunion of identical nuclei (A¹A¹, A²A², or a¹a¹, a²a²). Here, selection of pairs homozygous for *A* or for *a* would lead to an even greater distortion towards homozygosis for alleles at *B*. Unless both loci are closely linked to their centromeres, a 3 : 2 : 3 ratio of pairs would be expected for *B*. This ratio is demonstrated for the nuclear types A¹A¹ and A¹A²:

	BB	Bb	bb
.5 A¹A¹	.25	0	.25
.5 A¹A²	.125	.25	.125
	.375	.25	.375

Tests of hypotheses: Each of these hypotheses leads to specific genetic expectations for locus *A* and for locus *B* when *A* homozygotes are selected. In order to test which of these hypotheses is applicable, a particular type of cross was designed. A heterozygote between the A and B strains was utilized as the normal mate and mated to C*. The A/B cell is heterozy-

gous for *H* alleles (H^A/H^D) not contained in C*, which is H^E/H^E. The A/B cell is also heterozygous for alleles at the *P-1* locus ($P-1^A/P-1^B$), while C* is $P-1^B/P-1^B$.

In a normal cross of an A/B heterozygote and the C strain, a 1:1 segregation of H^A/H^E and H^D/H^E would be expected. For *P-1*, a 1:1 segregation of $P-1^A/P-1^B$ and $P-1^B/P-1^B$ would be expected. If, however, 2 meiotic products are contributed from A/B, the genetic expectations are different and depend upon which hypothesis is under consideration. The genetic expectations for *H* and for *P-1* are summarized in Table 6 for each of the 3 hypotheses.

TABLE 7. *Crosses of A/B × C*.*

	A/B	C*
a.	H^A/H^D	H^E/H^E
b.	$P-1^A/P-1^B$	$P-1^B/P-1^B$

a. Segregation of H alleles

Cross	Ex-conjugants separated	No. of pairs with phenotype: Ha	Had	Hd	Total	Hde
1.	No	21	39	30	90	1
2.	Yes	13	21	13	47	0
Total observed		34	60	43	137	
Expected 1:4:1		22.8	91.3	22.8	p <.0001	
Expected 1:3:1		27.4	82.2	27.4	p <.001	
Expected 1:2:1		34.25	68.5	34.25	p = .2	

b. Segregation of P-1 alleles in H homozygotes

	No. of pairs with phenotype: P-1A	P-1AB	P-1B	Total
Ha	9	7	9	25
Hd	11	6	8	25
Total observed	20	13	17	50
Expected 1:2:1	12.5	25	12.5	p <.01
Expected 5:6:5	15.6	18.8	15.6	p = .2
Expected 3:2:3	18.75	12.5	18.75	p = .8

The results of the cross of A/B and C* appear in Table 7. The H serotypes were screened in 138 pairs. Only 1 pair was formed by a normal conjugation. This pair, which was Hde, showed that C* did contribute H^E. All other pairs showed only segregation of the H^A and H^D alleles, contributed from the A/B heterozygote. The exconjugants from 47 of these pairs were tested separately. The H serotypes were iden-

TABLE 6. *Genetic expectations of A/B × C* under three hypotheses.*

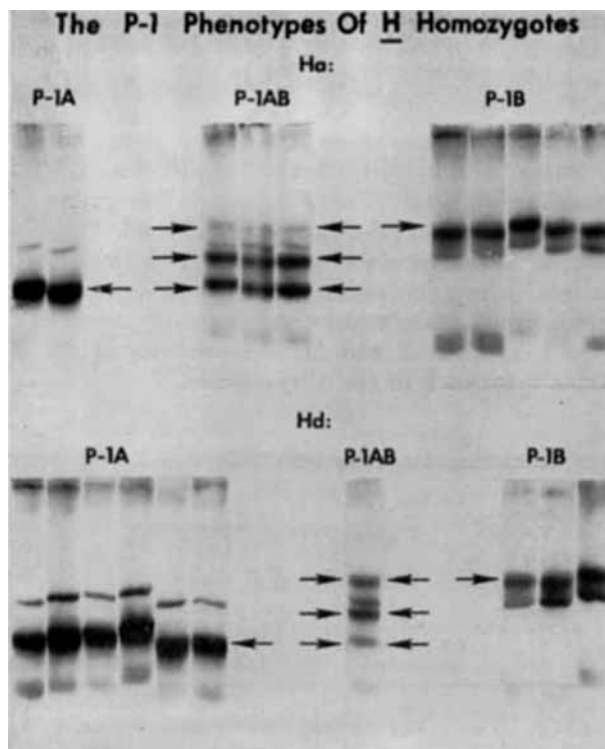|	Distribution of *H* pairs H^A/H^A	H^A/H^D	H^D/H^D	Distribution of *P-1* pairs among *H* homozygotes $P-1^A/P-1^A$	$P-1^A/P-1^B$	$P-1^B/P-1^B$
2 meiotic products from A/B						
Hypothesis 1	1 :	4 :	1	1 :	2 :	1
Hypothesis 2	1 :	3 :	1	5 :	6 :	5
Hypothesis 3	1 :	2 :	1	3 :	2 :	3

Fig. 1. Zymograms of the P-1 phosphatases of 10 Ha pair cultures (top) and 10 Hd pair cultures (bottom) from a cross of A/B × C*. The arrows indicate the electrophoretic positions of the P-1 phosphatases in the starch gels. The extracts were inserted into the starch at the origin which is at the top of each gel. The anode is at the bottom. The separations were achieved in 5 hours by an 8-9 v/cm drop in the starch gels made up in a boric acid-Tris buffer at pH 7.5. Strips of the starch gels were incubated in test tubes for 1 hour with sodium alpha-naphthyl acid phosphate as substrate and the diazonium salt of Fast Garnet GBC as dye-coupler at pH 5.0.

tical in the exconjugants of each pair. The observed distribution of pairs—34 Ha, 60 Had and 43 Hd, fits a 1 : 2 : 1 ratio (p = .2) and does not fit a 1 : 4 : 1 ratio (p<.0001), nor a 1 : 3 : 1 ratio (p<.001). The observations are thus compatible with only 1 of the 3 hypotheses: that is, Hypothesis 3.

Twenty-five Ha pairs and 25 Hd pairs were selected and screened for their P-1 acid phosphatases. Exconjugant cultures of 10 of these pairs, when examined separately, showed identical phenotypes for each pair. The observed distribution of pairs—20 P-1A, 13 P-1AB and 17 P-1B, fits a 3 : 2 : 3 ratio best (p = .8), but it also fits a 5 : 6 : 5 ratio (p = .2). It does not fit a 1 : 2 : 1 ratio (p<.01). The observations are thus compatible with either Hypothesis 3 or Hypothesis 2.

A photograph of the P-1 phenotypes of the first 10 pair cultures of each H type is shown in Fig. 1. This was a completely unselected group of cultures, although the order of the zymograms was changed in rearranging them into the 3 phenotypic classes. A

total of 8 P-1A, 4 P-1AB and 8 P-1B is demonstrated in these 20 cultures.

The combined observations on serotype and acid phosphatase distributions offer strong support for the 3rd hypothesis: that it, that a replica of 1 of the 4 meiotic products fuses randomly with 1 of them. This experiment provides evidence against the 1st and 2nd hypotheses, particularly against the 1st hypothesis. It also provides confirmatory evidence against the idea that C* induces illegitimate matings of normal cells, since a 1 : 2 : 1 segregation would be expected for alleles at *both* the *H* and *P-1* loci. Since the genetic ratios observed in this experiment conform best to the genetic expectations predicted for Hypothesis 3, a limit is imposed upon the possible cytogenetic schemes that can be considered.

Cytogenetic Bases for Genomic Exclusion

All the evidence points to the conclusion that in a cross of C* more than 1 meiotic product is contributed from the normal mate. Moreover, a special set of requirements is generated when the normal mate is a heterozygote. In order to account for the observed genetic distributions, a replica of 1 of the 4 meiotic products must fuse randomly with 1 of these nuclei. The requirements of the genetic theory are, therefore, rigorous enough to restrict the sequences of nuclear events that can be considered.

Two schemes meeting these requirements can be envisioned depending upon whether single or double fertilization occurs (Fig. 2). If all the prezygotic divisions (MI, II and III) took place in the normal mate and random fusion of 1 MIII product with 1 of the other 4 haploid nuclei occurred to produce a diploid nucleus, the diploid nucleus must divide and 1 of the products must migrate to C* in order to fulfill the requirement that the C* mate becomes diploid after mating. This is the "single fertilization" scheme outlined in Fig. 2.

Alternatively, C* could gain a haploid nucleus after the 3rd prezygotic division (MIIIa) by transfer from the normal mate; then, a 2nd mitotic division (MIIIb) could take place. In C* the transferred haploid nucleus could divide again. In the normal mate any 1 of the 4 haploid nuclei might undergo this division. The products of MIIIb might function as migratory and stationary nuclei, permitting reciprocal fertilization as in a normal mating. This is the "double fertilization" scheme outlined in Fig. 2.

A cytological study of C* during conjugation was initiated before the genetic basis of genomic exclusion was worked out. At the time we had no clear idea at all as to what to look for. Moreover, the techniques used in making the crosses resulted in a panorama of pairs in different stages of conjugation, making the
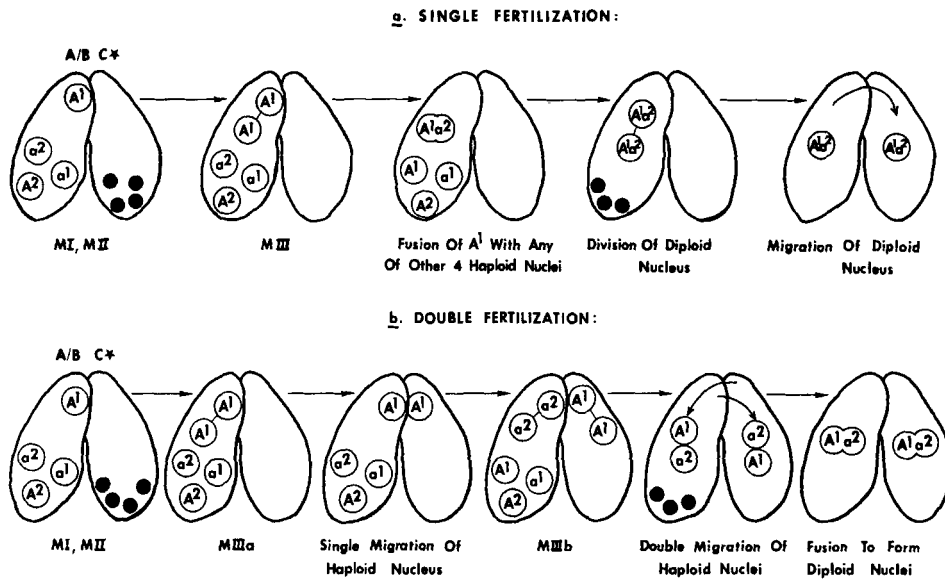
Fig. 2. Two schemes of possible sequences of nuclear events that might occur in an outcross of C*: (a) the single fertilization scheme; (b) the double fertilization scheme.

assessment of sequences in the stages exceedingly difficult. This was particularly true for the critical intermediate stages.

In this study the crosses were made in the Cerophyl-*Aerobacter* medium. Samples were removed at regular intervals (6, 12, 24, 48, 72 hours) and Feulgen preparations were made. In some of the crosses the C*GIANT subline was used to mark C*.

With this protocol new pairs form over the entire period of observation. However, we could conclude that the very early stages of conjugation were normal: crescents form in both mates and both mates undergo MI and MII. The products of meiosis seemed to disintegrate in C*. What could not be determined at that time was the extent to which intermediate stages occur in the C* cytoplasm. The stage that seemed to show irregularities was the 3rd prezygotic division. Normally, 1 of the products of the 2nd prezygotic division ends up in the paroral cone but in these crosses often 1 of the products failed to end up in this region in C*. In samples of pairs after 24 hours the 3rd prezygotic division seemed to be taking place in only the normal mate. In the 72-hour sample the 3rd prezygotic division occurred in both mates of some pairs. The late stages of conjugation were normal: the postzygotic divisions and macronuclear enlargement take place in both mates.

This study provided confirmatory evidence that both exconjugants end up with a normal complement of nuclei. However, at the time these observations were made, the peculiarities of the 3rd prezygotic division were very puzzling. In light of our present knowledge, the observation that some pairs did seem to undergo a 3rd prezygotic division in both mates

infers that the double fertilization scheme may be applicable. Before any definitive conclusions can be drawn, a more detailed analysis of these intermediate stages is needed. In repeating this study we plan to use a technique that will prevent new pairs from forming, making observation of the sequence of stages somewhat simpler.

The cytogenetic basis for genomic exclusion has, therefore, not yet been resolved. The alternatives are clearly defined so that it should be a relatively simple task to make a choice between them.

DISCUSSION AND CONCLUSIONS

C* provokes unilateral genomic exclusion in almost all pairs. This property was invaluable for making possible an analysis of the genetic basis of genomic exclusion. In a cross of C* 2 meiotic products are contributed from the normal mate. An experiment designed to test the genetic expectations of 3 different hypotheses was set up using as the normal mate a heterozygote containing alleles (H^A/H^D) not present in C*. The observed distributions were compatible with Hypothesis 3. This hypothesis states that 1 of the 4 meiotic products replicates and the replica fuses randomly with 1 of the 4 nuclei. The cytogenetic basis of genomic exclusion has not been determined, but the genetic requirements are sufficiently rigorous to limit the sequences of nuclear behavior that can be considered. These requirements can be fulfilled under 2 schemes depending upon whether single or double fertilization occurs.

A corollary of Hypothesis 3 is that ½ of the homozygotes for a single factor will be "pseudoau-

togamous," or homozygous for all their genes. Autogamy has never been observed in variety 1 of *T. pyriformis*. Thus, the appearance of "pseudoautogamy" is an interesting consequence of genomic exclusion and conceivably could be associated with the breakdown of an "outbreeding" economy during the course of inbreeding. This seems especially intriguing in view of Nanney's(10) observation that genomic exclusion tends to occur more frequently in stocks that are now in the 12th or 13th generation of inbreeding.

Other members of the C strain behave abnormally in outcrosses to a lesser degree and the types of distortions may be different. Some provoke unilateral genomic exclusion, but, instead of recovering markers from the other parent, only those of the C parent are recovered. Others provoke bilateral genomic exclusion. For example, a cross of B-8572 $\times$ C-6586 resulted in 22 normal pairs that were E-1BC, 3 E-1B pairs that were also Hd and 3 E-1C pairs that were He. These unusual pairs were testcrossed. Those that were E-1B were homozygous for $E\text{-}1^B$, $E\text{-}2^B$, mt^B and H^D, while those that were E-1C were homozygous for $E\text{-}1^C$, $E\text{-}2^C$, mt^C and H^E.

The same stock may behave somewhat differently when crossed to different "normal" mates. A series of mates of different genotypes were used in crosses to C-6586, all performed within an interval of 4 weeks. A cross to an F2 E-1BC mate resulted in a normal distribution of 13 E-1BC to 12 E-1C pairs, while a cross to the B strain resulted in the above distribution in which bilateral genomic exclusion was demonstrated. A cross to an F1 E-1BC led to the 1:2:1 ratio shown in Table 4, while a cross to another F1 E-1BC mate led to a 1 E-1BC to 19 E-1C distribution. Thus, C-6586 may behave normally in crosses to some mates; or, it may provoke unilateral genomic exclusion in crosses to some mates and bilateral genomic exclusion in crosses to other mates. The type of behavior that results may depend upon which "normal" stock is used.

Genomic exclusion is not confined to the C strain. D. L. Nanney(10) has found aberrant segregation in over half the crosses of highly inbred derivatives of several strains. He has observed both unilateral and bilateral genomic exclusion in these crosses. The frequency of genomic exclusion seems to be higher in crosses that are less than 50% viable and if crosses are made at temperatures other than "standard" (23-26°C). Some strains, like B1 and D, are particularly prone to behave abnormally. Nanney believes that the abnormalities in segregation may be a late manifestation of inbreeding degeneration. He points out that certain precautions will have to be adopted in carrying out the inbreeding program in order to in-

sure the vigor and genetic performance of the inbred stocks.

For most genetic work genomic exclusion is a most undesirable feature. Its presence has resulted in the discarding of a considerable amount of data, often obtained with much time and effort. However, under some special circumstances, genomic exclusion might be put to work in a positive manner. For example, a stock such as C* might be employed to derive "recombinant" stocks resulting from crosses to heterozygotes. Various combinations of genes, in homozygous form, could be extracted by selection of the "pseudoautogamous" lines. From a cross of A/B and C* we have been able to derive 7 different homozygous lines for combinations of alleles at the *H*, *P-1* and *mt* loci. Thus, in spite of its negative features, on rare occasions genomic exclusion might serve a useful purpose.

REFERENCES

1. Allen, S. L. 1960. Inherited variations in the esterases of Tetrahymena. *Genetics* 45, 1051-70.

2. ——— 1960. Abnormal segregation at the mating type locus in variety 1 of *Tetrahymena pyriformis*. *J. Protozool.* 7 (Suppl.), 15.

3. ——— 1961. Genetic control of the esterases in the protozoan *Tetrahymena pyriformis*. *Ann. N. Y. Acad. Sci.* 94, 753-73.

4. Allen, S. L., Misch, M. S. & Morrison, B. M. 1963. Variations in the electrophoretically separated acid phosphatases of Tetrahymena. *J. Histochem. Cytochem.* (in press).

5. ——— 1963. Genetic control of an acid phosphatase in Tetrahymena: formation of a hybrid enzyme. *Genetics* (in press).

6. Elliott, A. M. & Clark, G. M. 1956. The induction of haploidy in *Tetrahymena pyriformis* following X-irradiation. *J. Protozool.* 3, 181-8.

7. Larison, L. L. & Siegel, R. W. 1961. Illegitimate mating in *Paramecium bursaria* and the basis for cell union. *J. Gen. Microbiol.* 26, 499-508.

8. Loefer, J. B. & Owen, R. D. 1961. Characterization and distribution of "H" serotypes in 25°C cultures of *Tetrahymena pyriformis*, variety 1. *J. Protozool.* 8, 387-91.

9. Nanney, D. L. 1959. Genetic factors affecting mating type frequencies in variety 1 of *Tetrahymena pyriformis*. *Genetics* 44, 1173-84.

10. ——— 1963. Irregular genetic transmission in Tetrahymena crosses. *Genetics* 48, 737-44.

11. Nanney, D. L. & Caughey, P. A. 1955. An unstable nuclear condition in *Tetrahymena pyriformis*. *Genetics* 40, 388-98.

12. Nanney, D. L. & Dubert, J. M. 1960. The genetics of the H serotype system in variety 1 of *Tetrahymena pyriformis*. *Genetics* 45, 1335-49.

13. Nanney, D. L., Reeve, S. J., Nagel, J. & DePinto, S. 1963. H serotype differentiation in Tetrahymena. *Genetics* 48, 803-13.

14. Sonneborn, T. M., personal communication.