

A GENETIC ANALYSIS OF TETRAHYMENA THAT HAVE ABORTED NORMAL DEVELOPMENT

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ABSTRACT

Conjugating *Tetrahymena* can abort the sexual cycle before the production of progeny somatic (macro-) nuclei and return to vegetative growth. We have analyzed the germinal (micronuclear) genotypes of these cells in order to determine the stage at which they aborted conjugation. Our data demonstrate that nearly all of these cells attempt meiosis, but that very few reach the successful completion of cross-fertilization. The resulting micronuclear genotypes suggest that either germinal chromosomes or entire nuclei are lost during an unsuccessful attempt at meiosis or cross-fertilization. We conclude that the decision to develop progeny macronuclei is made during meiosis and is dependent on the completion of some step necessary for successful cross-fertilization.

CONJUGATION in the ciliated protozoan *Tetrahymena thermophila* may be a useful model for studying a simple nuclear differentiation. *Tetrahymena* is binucleate, having separate germinal and somatic nuclei: the transcriptionally silent, diploid micronucleus and the transcriptionally active, large (45C amount of DNA) macronucleus, respectively. The macronucleus is solely responsible for the phenotype of the cell (GOROVSKY and WOODWARD 1969; BRUNS and BRUSSARD 1974a). Conjugation results in the production of new, recombinant micro- and macronuclei. Both of these nuclei are mitotically derived from the same zygotic nucleus formed during conjugation (RAY 1956). The development of a new macronucleus involves both amplification and sequence rearrangement of the germinal genome (YAO and GALL 1979).

During conjugation, cells are confronted with a major developmental decision: after meiosis and the production of a new germinal nucleus, mating cells can either develop this new genome into a new somatic (macro-) nucleus or abort conjugation and return to vegetative growth. Cells that have aborted conjugation retain their parental macronuclei and therefore their parental phenotypes. These cells have been termed nonconjugants by NANNEY (1957).

In a previous paper (SCHOLNICK and BRUNS 1982), we demonstrated that nonconjugants can be selected by allowing the cells to mate at 40°. This treatment is lethal to all cells producing new macronuclei and only the nonconjugants survive (SCHOLNICK and BRUNS 1980; SCHOLNICK and BRUNS 1982). In this paper we demonstrate that the nonconjugants selected from 40° matings are genetically similar if not identical to their 30° nonconjugant counterparts. We have found that nonconjugation is associated with a limited set of genetic

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abnormalities almost always associated with an early step in conjugation. These results reveal both the timing of, and a possible mechanism for, the events leading to the subsequent abortion of macronuclear development.

MATERIALS AND METHODS

Strains: The strains used in this study are fully described in Table 1. All of these strains are derived from inbred strain B1868. SB210 was provided by Dr. EDUARDO ORIAS, University of California, Santa Barbara. *T. thermophila* was formerly known as *Tetrahymena pyriformis*, syngen 1 (NANNEY and MCCOY 1976).

Media: The growth medium (PPYS) was 1% proteose peptone (Difco), 0.1% yeast extract (Difco), and 0.003% Sequestrene (Geigy). The starvation medium was 10 mM Tris-HCl, pH 7.4 (Sigma). Starved cells were fed with 10% proteose peptone to a final concentration of 1%. Stocks of strains were maintained in 1% proteose peptone at room temperature. All media were sterilized by autoclaving.

Matings in flasks: As previously described (BRUNS and BRUSSARD 1974b) cells were prepared for mating by washing once in 10 mM Tris and resuspending in Tris at a final concentration of 1.4×10^6 cells/ml. Cells were allowed to starve for 18-24 hr. Matings were made by mixing equal numbers of the prestarved parents in an Erlenmeyer flask at a final concentration of 1.4×10^6 cells/ml. Cell numbers were determined using a Coulter Counter (Coulter Electronics) with a 200- μ m aperture. The flasks used to contain the matings had a capacity of at least 10 times the volume of the mating mix. Unless otherwise noted, the matings were fed 6 hr after mixing in order to stop further pairing.

Pair isolation, subcloning and microtiter plate manipulation: Pairs were isolated by micropipette starting 30 min after refeeding the mating mix. Pairs were transferred to individual drop cultures arranged in 6 x 8 array in a 100- x 15-mm disposable Petri dish. This array matches the pattern of wells found on microtiter plates (Dynatech; Costar). The pairs were allowed to grow for 3 days at 30° or 40°. They were then transferred to microtiter plates by using a custom 48-prong stainless steel replicator (Lansing Industries). These master plates were replicated to other plates for drug testing or further matings by using a 96-prong stainless steel replicator (Lansing Industries). Subclones of the strains used in these experiments were established by isolating single cells from vegetative cultures. These cells were manipulated in the same manner as isolated pairs.

Matings in microtiter plates: Microtiter plate cultures were grown overnight at 30° in 0.1 ml/well PPYS. All of the cultures on the plate were pelleted by gentle centrifugation in an IEC model UV centrifuge equipped with microtiter plate carriers (Dynatech). The medium was removed with a custom-made 96-channel aspirator. The cells were resuspended in 0.1 ml/well 10 mM Tris using a 12-channel manifold (Dynatech) and a Cornwall repeating syringe. The cultures were pelleted once more and resuspended in 0.05 ml/well 10 mM Tris. These cultures were allowed to starve overnight at 30°. Mating was initiated by adding 0.05 ml/well of a prestarved culture of the appropriate strain. Matings used for the isolation of pairs were fed with 5% proteose peptone to a final concentration of 1% peptone 6-8 hr after mixing the parents. Matings used for mating-type testing were not refed; 6-8 hr-old matings were examined for the presence of pairs, using a dissecting microscope with darkfield illumination.

Drug selection: The drug doses used for selecting cycloheximide- and 6-methylpurine-resistant cells were 25 μ g/ml and 15 μ g/ml, respectively. These drugs were maintained as 500 \times stocks in 95% ethanol or distilled water, respectively, at -20°. The dose used for selecting 2-deoxygalactose-resistant cells was 2.5 mg/ml. This drug was maintained as a 50 \times stock in distilled water at -20°. All three drugs were diluted with PPYS just before use. Cells were maintained in the drug media for 4-5 days (cycloheximide and 6-methylpurine) or 7-8 days (2-deoxygalactose) before resistance was scored.

RESULTS

Experimental rationale: We compared the genetic consequences of nonconjugation at 30° and 40° by analyzing the micronuclear genotypes of nonconjugants collected from the cross *Mpr/Mpr+* (6mp-s) \times *Chx/Chx* (cy-s) (CU326 \times CU329 or CU330). All of these strains were heterokaryons for drug resistance alleles as described in Table 1. Although these strains carry germinal mutations

TABLE 1
Strains used in this study

Designation	Genotype (phenotype) ^a
CU326	<i>Mpr/Mpr+</i> (6mp-s V)
CU329	<i>Chx/Chx</i> (cy-s II)
CU330	<i>Chx/Chx</i> (cy-s IV)
CU336	<i>Mpr/Mpr</i> (6 mp-s VII)
CU355	<i>Chx/Chx</i> (cy-s IV)
SB210	<i>gal/gal</i> (2dgal-s VI)

^a As previously suggested (BRUNS and BRUSSARD 1974a), the letters preceding the parentheses designate the micronuclear genotype of the cell; the letters inside the parentheses represent the phenotype expressed by the macronucleus, and the Roman numerals stand for the mating type. *Chx* is a dominant mutation conferring resistance to cycloheximide (BYRNE, BRUSSARD and BRUNS 1978). *Mpr* is a dominant 6-methylpurine resistance mutation (BYRNE, BRUSSARD and BRUNS 1978). *gal* is a recessive mutation conferring resistance to 2-deoxygalactose (ROBERTS and MORSE 1980). CU329 is therefore a heterokaryon with a germline homozygous for the *Chx* mutation. The cell's phenotype is cycloheximide sensitive and its mating type is II. See BRUNS and BRUSSARD (1974a) for a description of how these strains were constructed.

conferring resistance to either 6-methylpurine or cycloheximide, their macronuclei express the wild-type drug-sensitivity phenotype. The parents and nonconjugants are therefore sensitive to both drugs. All of the progeny are resistant to cycloheximide and one-half are resistant to 6-methylpurine. We assayed the micronuclear genotypes of the clones derived from each parent of the nonconjugant pairs (termed the exconjugant clones) by a testcross to SB210, a heterokaryon with a micronucleus homozygous for the *gal* mutation. This strain is a homozygous homokaryon for the wild-type *Chx+* and *Mpr+* alleles (Table 1) (ROBERTS and MORSE 1980).

The data generated by this experimental approach allowed us to test the validity of our temperature selection for nonconjugants and to gain some understanding of the mechanism of the developmental decision to abort macronuclear differentiation.

Genetic analysis of the nonconjugants' micronuclear genotypes detects the types of genetic abnormalities, if any, associated with nonconjugation. This allows a first approximation of the step in the pathway at which the cells abort conjugation. Conjugation before macronuclear development can be divided into three distinct periods: 1) premeiosis, 2) postmeiosis-precross-fertilization and 3) postcross-fertilization.

These three periods are diagrammed in Figure 1. Each potential stopping point in conjugation is associated with its own distinctive set of predictions for the nonconjugants' micronuclear genotypes. These predictions are listed in Table 2. The two cells of each nonconjugant pair will differ in their micronuclear genotypes except in cases in which the pair aborts conjugation after cross-fertilization (Table 2). We were able to distinguish between the exconjugant

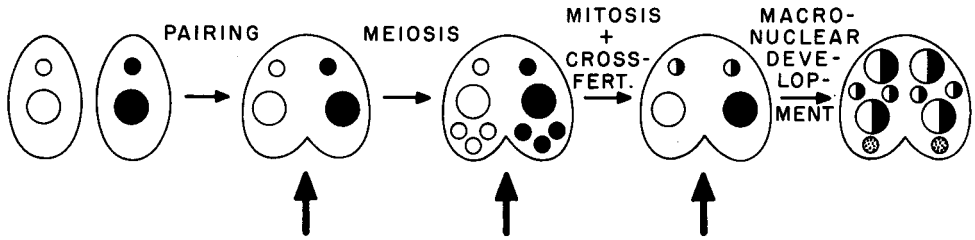


FIGURE 1.—Schematic showing the major events of conjugation. The heavy black arrows indicate the three genetically distinguishable stopping points as outlined in the text (*Experimental rationale*). The diagram shows a cross between two homozygous homokaryons.

TABLE 2
Predicted micronuclear genotypes of nonconjugants

Stopping point/aberration ^a	Exconjugant clones	
	Mating type V ^b	Mating type II or IV ^c
Premeiosis	<i>Mpr/Mpr+</i>	<i>Chx/Chx</i>
Postmeiosis/pre-XF ^d	<i>Mpr/Mpr</i>	<i>Chx/Chx</i>
	<i>Mpr+/Mpr+</i>	or
Post-XF	<i>Mpr/Mpr+</i>	<i>Mpr/Mpr+</i>
	<i>Chx/Chx+</i>	<i>Chx/Chx+</i>
		or
	<i>Mpr+/Mpr+</i>	<i>Mpr+/Mpr+</i>
	<i>Chx/Chx+</i>	<i>Chx/Chx+</i>
Genomic exclusion	<i>Mpr/Mpr</i>	<i>Mpr/Mpr</i>
	<i>Chx+/Chx+</i>	<i>Chx+/Chx+</i>
		or
	<i>Mpr+/Mpr+</i>	<i>Mpr+/Mpr+</i>
	<i>Chx+/Chx+</i>	<i>Chx+/Chx+</i>
		or
	<i>Mpr+/Mpr+</i>	<i>Mpr+/Mpr+</i>
	<i>Chx/Chx</i>	<i>Chx/Chx</i>

^a See Figure 1.

^b e.g., derived from CU326 (Table 1).

^c e.g., derived from CU329 or CU330 (Table 1).

^d XF = cross-fertilization.

clones because they have retained their parental macronuclei and therefore their parental mating types.

The design of these experiments also allowed us to detect another cytogenetic event potentially associated with nonconjugation. The highly aneuploid star strains described by ALLEN (1967) are, in a sense, unconditional developmental mutants which induce nonconjugation in crosses to normal, euploid strains (termed Round I of genomic exclusion). These Round I nonconjugants are micronuclear whole genome homozygotes. These micronuclear genomes are derived solely from the gametic nucleus of the normal, nonstar parent. The Round I exconjugants can remate (Round II of genomic exclusion) and produce new macronuclei expressing the homozygous genotypes generated during

Round I. If a Round I genomic exclusion-like event is associated with nonconjugation in wild type crosses it would result in a class of nonconjugants with its own predictable and distinct set of micronuclear genotypes. These are also listed in Table 2.

Collection of the nonconjugants: CU326 and either CU329 or CU330 (Table 1) were grown and starved separately and then mixed for mating as described in MATERIALS AND METHODS.

Nonconjugants were collected from 30° matings in the following fashion. The mating cells were fed 6 hr after mixing the prestarved parents and pairs were isolated in drops of growth medium (PPYS) in Petri dishes (MATERIALS AND METHODS). These cultures were incubated at 30° for 3 days and then replicated to microtiter plates containing PPYS medium. After 3 days of growth these master plates were replicated to media containing cycloheximide and 6-methylpurine. The parental strains are sensitive to both drugs. Half of the progeny are resistant to 6-methylpurine and all are resistant to cycloheximide. Nonconjugant pairs are those that did not produce new, progeny macronuclei. They give rise to clones that have retained their parental mating type and drug-sensitive phenotype. Their status as nonconjugants was confirmed in each case by the fact that these cells were still capable of mating (mature) and still expressing their parental mating type. Tetrahymena which have produced new macronuclei are incapable of mating for approximately 40 fissions (NANNEY and CAUGHEY 1953).

The nonconjugants identified by this procedure were subcloned by isolating single cells from the master culture. These subclones were tested for mating type by crossing them to the parental strains (MATERIALS AND METHODS), allowing us to identify the two exconjugant clones from each pair. It was not always possible to recover both exconjugant clones. This may result from the death of one of the exconjugants during conjugation or to one of the exconjugant clones outgrowing the other during subsequent vegetative growth. The exconjugant clones were transferred to stock tubes and saved for further analysis.

Nonconjugants were isolated from 40° matings in a similar fashion. CU326 and CU330 were grown, starved and mated at 30°. The mating mixes were fed at either 4.5 or 5.5 hr and pairs were isolated into drop cultures as above. These drop cultures were transferred to 40° at 5.5 or 7.5 hr (designated series 40°E and 40°L, respectively) and incubated at that temperature for 3 days. We have previously demonstrated that only pairs that have aborted conjugation before macronuclear development can survive this treatment (SCHOLNICK and BRUNS 1980; SCHOLNICK and BRUNS 1982). The survivors of these crosses were subcloned and tested for mating type in the same fashion as their 30° counterparts. Their nonconjugant nature was confirmed in every case by their maturity and the retention of their parental mating types. Exconjugant clones descended from each member of the nonconjugant pairs were transferred to stock tubes as before. We were again unable to obtain both exconjugant clones in all cases.

Genetic analysis of the nonconjugants: All of the nonconjugant clones were prepared for genetic analysis by transferring the stocks to individual wells on a microtiter plate. Three replicates of each master plate were prepared for mating as described in MATERIALS AND METHODS. Two of these replicates were mated to the parental strains in order to confirm the mating type and, therefore, the origin of the clones. The clones on the remaining plate were crossed to

SB210, a heterokaryon for the *gal* mutation (ROBERTS and MORSE 1980), which confers resistance to 2-deoxygalactose (Table 1); 44 pairs were isolated from each of the testcrosses. These pairs were grown at 30° for 3 days and then replicated to microtiter plates. These master plates were incubated for 3 days and then replicated to media containing 2-deoxygalactose, cycloheximide or 6-methylpurine in order to identify the testcross progeny and ascertain their phenotypes.

Table 3 lists the results obtained from the analysis of only those nonconjugants for which we recovered both exconjugant clones. The analysis of those nonconjugants represented by only one exconjugant clone is consistent with these results (data not shown). However, the recovery of only one of the exconjugant clones does not allow the resolution of certain types of cytogenetic events: the recovery of a mating type II nonconjugant clone (i.e., one derived from CU329) with the micronuclear genotype *Chx/Chx Mpr+/Mpr+* could have been the result of three possible cytogenetic events: abortion of conjugation before meiosis, after meiosis but before cross-fertilization, or genomic exclusion (Table 2).

Although some pairs were recovered in each category, by far the most common class (64.4% of the nonconjugants recovered) appeared to be the result of a process similar to Round I genomic exclusion. Thus, in most cases both of the exconjugant clones from a given pair are homozygous for the marker inherited from only one of the parents (Table 2). The genome of the *Mpr* heterozygote, CU326, was recovered more frequently than that of the *Chx* homozygotes, CU329 and CU330 (31:9). The *Mpr* and *Mpr+* alleles segregated with a 1:1 ratio (15 *Mpr*:16 *Mpr+*) when all of the genomic exclusion events are taken into account.

We also recovered four nonconjugant pairs that appear to have undergone a self-fertilization. This is indicative of the abortion of conjugation after meiosis but before cross-fertilization (Tables 2 and 3). An additional nine pairs did not fit any of the predicted classes described above nor could they be reconciled with the normal segregation of meiotic products (Table 3). The micronuclear genotypes of these pairs are listed in Table 4.

Origin of the genomic exclusion events: The genomic exclusion events asso-

TABLE 3
Distribution of nonconjugant classes

Stopping point/aberration ^a	Treatment		
	30°	40°E ^b	40°L ^c
Premeiosis	NR ^e	NR	3
Postmeiosis/pre-XF	NR	2	2
Post-XF	1	NR	NR
Genomic exclusion	16	15	9
Abnormal segregation ^d	2	3	4
Infertile	2	1	2
Total	21	21	20

^a See Figure 1 and Table 2.

^b Transferred to 40° at 5.5 hr.

^c Transferred to 40° at 7.5 hr.

^d See text.

^e NR = not recovered.

TABLE 4
Micronuclear genotypes of clones showing abnormal segregation

Source ^a	Exconjugant clones ^b	
	Mating type V ^c	Mating type II or IV ^d
30°	Chx/Chx+ Mpr+/Mpr+	Chx/Chx Mpr+/Mpr+
30°	Chx+/Chx+ Mpr/Mpr	Chx+/Chx+ Mpr/Mpr+
40°E	Chx+/Chx+ Mpr/Mpr	Chx/Chx+ Mpr+/Mpr+
40°E	Chx+/Chx+ Mpr/Mpr+	Chx+/Chx+ Mpr/Mpr+
40°E	Chx/Chx Mpr+/Mpr+	Chx+Chx+ Mpr/Mpr
40°L	Chx+/Chx+ Mpr/Mpr	Chx+/Chx+ Mpr+/Mpr+
40°L	Chx/Chx Mpr+/Mpr+	Chx+/Chx+ Mpr+/Mpr+
40°L	Chx/Chx Mpr+/Mpr+	Chx+/Chx+ Mpr/Mpr
40°L	Chx+/Chx+ Mpr/Mpr	Chx+/Chx+ Mpr/Mpr+

^a See text.

^b Each line in the table represents both exconjugant clones derived from a single nonconjugant pair.

^c e.g., derived from CU326 (Table 1).

^d e.g., derived from either CU329 or CU330 (Table 1).

ciated with nonconjugation could be caused by star-like cells within the parental strains. Alternatively, genomic exclusion could be the result of a cytogenetic accident during mating between normally fertile cells. We differentiated between these possibilities by subcloning CU329 and testing the fertility of these subclones in mass crosses to CU336, a homozygous heterokaryon for the *Mpr* mutation (Table 1). Fertile subclones of CU329 will yield progeny resistant to both cycloheximide and 6-methylpurine. Star-like subclones will yield exconjugants either sensitive to both drugs (as in Round I genomic exclusion), or resistant to only 6-methylpurine (as in Round II genomic exclusion). Completely infertile subclones will also yield exconjugants sensitive to both drugs.

Subclones of CU329 were established by cell isolation as described in MATERIALS AND METHODS. Replicates of these subclones were transferred to microtiter plates and mated to CU336 (MATERIALS AND METHODS). These matings were allowed to proceed for 24 hr at 30°, which is sufficient time for both rounds of genomic exclusion. This allowed us to distinguish between star-like and completely infertile subclones. The mating mixtures were then fed and allowed to grow at 30° for 1 day. They were then replicated to media containing cycloheximide and 6-methylpurine.

Fertility tests of 257 subclones of CU329 did not detect any star-like or infertile clones by the criteria described above. A retest of 258 new subclones approximately 8 mo later revealed the existence of 25 star-like clones, indicating that the breeding performance of CU329 is degenerating. We also tested 258 subclones of CU326 during this second set of fertility tests by crossing them to CU355, a homozygous heterokaryon for the *Chx* mutation. We did not detect any star-like clones in these tests.

DISCUSSION

The data presented in Table 3 indicate the nonconjugants recovered from matings transferred to 40° at 5.5 hr (series 40°E in Table 3) are similar if not

identical to those recovered from 30° controls and from matings transferred to 40° at 7.5 hr (series 30° and 40°L in Table 3). This suggests that the elevated temperature does not affect cross-fertilization, which occurs at approximately 6 hr after mixing (MARTINDALE, ALLIS and BRUNS 1982). We did not recover all classes of nonconjugants in all three treatments, possibly because of the small sample sizes involved. A problem in this study was the isolation of a sufficient number of nonconjugants from the 30° matings. We optimized our procedure for collecting nonconjugants at both temperatures by using older heterokaryons: CU326, CU329 and CU330. Although these strains are still fertile, crosses between them yielded more nonconjugants than crosses between younger strains, e.g., CU336 × CU355 (approximately 10% and 3% of the pairs isolated, respectively). The ability to select for nonconjugants directly should allow a much more extensive analysis of nonconjugation using a greater variety of strains.

These data also indicate that the vast majority of nonconjugants that were tested (54/57, since 5/62 were infertile and thus untestable) aborted conjugation at some step after the onset of meiosis but before the fusion of the gametic nuclei. This suggests that in many cases the decision to abort conjugation may be associated with aberrant micronuclear events before the completion of cross-fertilization. These data also suggest that almost all pairs are committed to attempt meiosis.

Four of the pairs examined appear to have undergone a self-fertilization similar to the micronuclear events of osmotically-induced cytogamy (ORIAS, HAMILTON and FLACKS 1979). Both of these variations of normal conjugation generate two different whole-genome homozygotes from each pair. However, elements of osmotically-induced cytogamy must be different from that of nonconjugation as the former results in the development of progeny macronuclei.

Most of the nonconjugants (64.4%) appear to have undergone genomic exclusion, a cytogenetic maneuver normally associated with aged or senile strains of *Tetrahymena* (ALLEN 1967). The fertility tests carried out on the parental strains at the same time as the collection and analysis of these nonconjugants failed to detect the existence of aged clones capable of constitutively inducing genomic exclusion. Genomic exclusion may therefore represent an aberrant cytogenetic pathway followed occasionally in wild-type crosses and constitutively in crosses to star strains. Our subsequent recovery of star-like clones on retesting CU329 8 months later is consistent with the published observation that such clones arise within ageing cultures (NANNEY 1957; PITTS 1979). The age-dependent mitotic mistakes that presumably give rise to the aneuploid star strains may be preceded by a period of increasing meiotic instability during which an increasing number of mating cells are induced to abort conjugation. Further degeneration of the clone would presumably yield cells which constitutively induce genomic exclusion. CU329 and CU330 acted as the star-like parent more often than CU326 (31:9). This is consistent with the hypothesis that CU329 and presumably CU330 are more senile than CU326. This is supported by our inability to detect star-like subclones of CU326 even in the second round of fertility testing. Although all three strains are of approximately the same chronological age, they appear to be ageing genetically at different rates. NANNEY

has suggested that germinal ageing is a consequence of the occurrence of random defects in the micronucleus and that the rate of ageing can vary from strain to strain (NANNEY 1974; SIMON and NANNEY 1979).

The restricted range of micronuclear genotypes detected in this study suggests that several classes of nonconjugants may be the result of similar cytogenetic events. Both the cytogamous micronuclear events and genomic exclusion may involve the loss of one or more of the gametic nuclei. It is possible that the decision to produce a new macronucleus is in part dependent on the successful completion of some event leading to cross-fertilization or to the successful completion of cross-fertilization itself. The former possibility seems to be the more likely of the two as the osmotic induction of cytogamy does not lead to nonconjugation (ORIAS, HAMILTON and FLACKS 1979). This hypothesis is consistent with our recovery of the class of nonconjugants labeled "abnormal segregation" in Table 4. The micronuclear genotypes of these pairs are not consistent with normal meiotic segregation of markers or with any of the cytogenetic abnormalities discussed above. However, these genotypes could be generated by the loss of one or more of the gametic nuclei after nuclear transfer but before the fusion of the nuclei. Alternatively, some of these micronuclear genotypes could be the result of the loss of one or more of the genetically marked germinal chromosomes. A strain monosomic for a dominantly marked germinal chromosome would behave like a heterozygote in a testcross to a diploid strain, i.e., one-half of the progeny would express this dominant phenotype and one-half would not. Such monosomic cells could arise as a result of an aberrant meiosis. However, monosomy is not sufficient to cause the cells to abort conjugation. Monosomic and even nullisomic strains are capable of completing conjugation when crossed to a diploid strain (BRUNS and BRUSSARD 1981). We have not karyotyped our collection of nonconjugants to see if chromosome loss is associated with nonconjugation. The infertile nonconjugant pairs listed in Table 3 might also result from meiotic accidents. In this case part of the germinal genome might have sustained much greater damage. An extreme possibility is that these strains have haploid micronuclei, so the production of progeny is below the resolution of these experiments.

Ample precedent exists for the involvement of cytogenetic abnormalities with nonconjugation. Meiosis in the classic, highly aneuploid, star strains, A*III, A*V, and C*III, appears to result in the loss of the micronucleus (ALLEN 1967; WEINDRUCH and DOERDER 1975). PITTS (1979) has reported several star strains that complete meiosis but fail to associate a meiotic product with the attachment membrane between the members of the pair; all of the meiotic products are subsequently destroyed. Random accidents of this nature during the conjugation of otherwise normal strains could be responsible for some of the abnormalities we have detected and for many incidents of nonconjugation. Our demonstration that high temperature matings faithfully select for nonconjugants provides an efficient way of assaying the number of cells in a mating mix that have aborted conjugation. This should allow the experimental dissection and manipulation of this developmental decision in a manner not previously possible.

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