IRREGULAR GENETIC TRANSMISSION IN TETRAHYMENA CROSSES[1]

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ALLEN (1960) reported briefly an aberration in the breeding behavior of a strain (C*) of variety 1 of *Tetrahymena pyriformis*. Her later studies (ALLEN 1963) established the genetic parameters of the aberration and lead to the conclusion that the total genome of the C* strain is systematically excluded at conjugation. Genomic exclusion does, however, involve a complete reorganization process and recombination of markers present in the normal parent if it is heterozygous. The occurrence of genomic exclusion in crosses to C* raises the possibility that similar aberrations may occur in other crosses and may bias breeding results in important ways.

During the past few years many crosses have been made among group of strains of variety 1 in a study of the genetic basis for antigenic differences. Several of these crosses have provided indications of genetic anomalies similar to those reported by ALLEN. The purpose of this report is to document the occurrence of genetic aberrations and to indicate some of the methods whereby crosses may be screened.

MATERIALS AND METHODS

The strain employed have all been inbred lines of variety 1 maintained by periodic crosses at least once each year. We are concerned here primarily with the transmission of the *mt* and *H* genes, and the genetic constitutions of the pertinent strains with regard to these factors are listed in Table 1 (see NANNEY 1959; NANNEY and DUBERT 1960).

TABLE 1

Genotypes of inbred strains studied

Inbred series	*mt* genotype	*H* genotype
A	mt^A/mt^A	H^A/H^A
A1	mt^A/mt^A	H^D/H^D
B	mt^B/mt^B	H^D/H^D
B1	mt^B/mt^B	H^C/H^C
C	mt^C/mt^C	H^E/H^E
D	mt^D/mt^D	H^D/H^D
D1	mt^D/mt^D	H^C/H^C

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Crosses were made on Cerophyl-Aerobacter medium by procedures previously described (NANNEY and CAUGHEY 1955; NANNEY, CAUGHEY and TEFANKJIAN 1955). Pairs were isolated but exconjugants were not separated; instead, two or three single cells were usually isolated from each pair-culture after ten or more fissions. All clones were carefully examined for "non-conjugation" and all lines which failed to become sexually immature at conjugation were eliminated (NANNEY, CAUGHEY and TEFANKJIAN 1955; NANNEY 1963). The clones were maintained in depression cultures by periodic single-cell transfers and were tested for serotypes after four or more transfers. Some were also tested for mating types when they became sexually mature after about ten transfers. Progeny tests were carried out by crossing two sister lines from the same pair to yield the F_2.

RESULTS

Anomalies have been detected in a variety of crosses, but they are established in different ways in different cases. We will first consider a series of illustrative examples and return later to a more general discussion of the distribution of aberrations.

Example 1: Cross #60-204. Parental strains: A and B1 at 26°C. A total of 82 sublines from 62 pairs were carried to maturity and typed. Of these, 80 were serotype Ha and two were serotype Hac. The heterozygote H^A/H^C generated by this cross, like other serotype heterozygotes (NANNEY and DUBERT 1960; NANNEY, unpublished), "differentiates," i.e., some sublines manifest only the Ha phenotype, some the Hc and a progressively smaller fraction continues to manifest both. The existence of two Hac sublines indicates that some of the progeny are heterozygous. The bias in the "output ratio," the relative predominance of the Ha over the Hc phenotype in the progeny, is not critical evidence for the exclusion of the B1 genome, since the Hc phenotype might be simply suppressed. However, in most heterozygous H^A/H^C populations the Ha phenotype, though the predominant type, comprises only 80-90 percent of the total. Hence, a suspicion of exclusion is raised by the serotype phenotypes.

More critical information comes from a tabulation of mating types. The mt^A gene (like the mt^C and mt^D genes) potentiates the development of any of five different mating types—I, II, III, V and VI. The mt^B gene potentiates six mating types—II, III, IV, V, VI and VII. Any one mature line ordinarily expresses only one of these types. The mt^A/mt^B heterozygotes generate a characteristic array of mating types and type IV usually makes up 20 percent or more of the total (NANNEY *et al.* 1955; NANNEY 1959). Of the 62 lines typed, only one was mating type IV, and this line was also one of the two lines which showed the Hac phenotype. No type VII lines were found. One must conclude, therefore, that the outputs of both mating types and serotypes are seriously disturbed in this cross; the genes of the B1 parent, if transmitted, are not expressed as frequently as they are in other crosses.

Example 2: Cross #61-80. Parental strains: A and C at 32°C. Three lines were initially isolated from each of 40 pairs, and 111 cultures were carried to

maturity. When tested for serotypes, ten lines were Ha and 101 were He. This output ratio is not unusual for H^A/H^E heterozygotes and does not cast suspicion on the legitimacy of the cross. A qualitative test for mating type arrays in this particular instance is also not critical. The genes mt^A and mt^C potentiate the same arrays of mating types—I, II, III, V and VI. However, the mating type frequencies are different in strains homozygous for the two alleles. Specifically, type I appears in about 20 percent of the mt^A homozygotes and near 50 percent in mt^C homozygotes. The fact that 53 of the 106 lines typed for mating type were mating type I suggested a bias toward the mt^C frequencies, possibly indicative of an exclusion of the mt^A allele. The criterion of mating type frequencies is not a powerful one, however, since it can be employed only in certain crosses and is decisive only when a reasonably large number of progeny is available.

In this particular example another criterion of more general applicability was used. Studies now in progress demonstrate very little correlation between the serotype outputs of sister caryonides. Since each pair produces four caryonides, the practice of isolating two or three lines from a pair culture generates a high probability of obtaining representatives of two or three caryonides. Particularly since nearly all caryonides yield mixed outputs, very little correlation in serotype is expected from the sister lines taken from the same pair, and in most crosses no such correlation is found. Yet, in this cross a complete correlation was obtained. All the ten sublines showing Ha were obtained from four pairs (two sublines from these were lost). In no cases were Ha and He specificities detected among the progeny of a single pair. Hence, on largely qualitative grounds, the pairs in the cross are shown to be heterogeneous. This heterogeneity is suggestive of bilateral genomic exclusion; in some pairs the A genome appears to be excluded and in a much smaller number of pairs the C genome is not detected.

Example 3: Cross #60-202. Parental strains B and C at 26°C. From this cross a total of 87 sublines were serotyped to yield 26 Hd, one Hde and 60 He. These results are not obviously aberrant except that the number of lines with intermediate phenotype is somewhat below that usually encountered at 100 fissions. The mating type array for types I to VII was 8:15:6:18:0:9:0 for the 53 lines typed; while this array does not differ appreciably from that expected in a population of mt^B/mt^C heterozygotes, it might also be produced by a population containing both mt^B and mt^C homozygotes. The mating type data indicate, therefore, that both parents contributed genes to the progeny, but bilateral genomic exclusion is not disproved.

The criterion of serotype heterogeneity, on the other hand, did demonstrate that something was abnormal. Twenty-five of the pairs in this cross were represented by at least two sublines. These pairs could be classified into three groups depending upon whether two sublines randomly selected manifested only Ha reactions, only He reactions, or both. If all the sublines manifested pure types, and if the serotypes of sister lines were uncorrelated, the classes of pairs should be predicted by the binomial expansion, $(p + q)^2$, where p is the

frequency of type Hd and $q(=1-p)$ is the frequency of type He. In this particular example, $p = 0.3$ and $q = 0.7$; the expanded binomial has the values of 0.9, 0.42 and 0.49. Applied to the sample of 25 pairs, the expected distribution is 2.25:10.5:12.25. The observed distribution of 7:2:17 obviously deviates from expectation; too few pairs are observed in the intermediate category; the pairs are not homogeneous.

This heterogeneity between pairs produced in a cross between two presumably homozygous strains does not *a priori* demand a genetic explanation, since an epigenetic alternative is also plausible. On the other hand, most crosses do yield pairs which are homogeneous with this test and the occasional crosses which manifest heterogeneity must have some special explanation. Bilateral genomic exclusion may well be the explanation.

Simply to illustrate the application of this test when homogeneity is encountered we may consider briefly cross #61-20, between inbred strains A and C. A total of 90 sublines were classified as follows: 11 Ha, ten Hae, 69 He. The calculated "output ratio" (counting the lines with mixed reactions as half Ha and half He) was 18 Ha:82 He. Twenty-nine pairs were represented by three sublines. Of these, none expressed only Ha, sixteen manifested both specificities and thirteen pairs manifested only He. The frequencies predicted from the expanded binomial $(p+q)^3$ are 0:13:16. The slight excess in the intermediate category is commonly found and reflects the fact that all the sublines have not completely differentiated; a pair may be assigned to a mixed category on the basis of a single subline.

Example 4: Cross #60-188. Parental strains C and D at 26°C. A total of 93 sublines were classified as one Hd, three Hde and 89 He. The mating type distribution was 35:9:6:0:2:30:0 for 82 lines, and was not decisive. The serotype distribution was too eccentric to provide a binomial test. In such cases the only remaining test possible is a progeny test, but this is a tedious process to carry out on each of the F_1 pairs, and only spot checks were made. In this case two pairs manifesting only He were inbred separately by crossing sister lines of different mating type. From one of these crosses 88 sublines were derived and all were He; from the other cross only 22 lines were examined, but they again were all He. At least one fourth of all the F_2 pairs should have produced exclusively Hd progeny, but no Hd lines were recovered. Hence, at least some of the F_1 pairs received no *H* gene from the D parent.

Correlates of irregular transmission. In the preceding section various techniques used to screen crosses for irregularities in genetic transmission are illustrated. The examples demonstrate that the irregularities are not restricted to crosses with the anomalous C* strain, but may occur in crosses with a variety of strains. These screening techniques were applied to 48 crosses carried out over a three year period. Of these crosses only 23 were unambiguously normal by the available techniques. The other 25 crosses were either clearly abnormal (16) or provided some grounds for suspicion (nine). The crosses included in this analysis are listed (Table 2) with fractions judged to be abnormal. Irregular

TABLE 2

Crosses analyzed with indications of fractions aberrant

		Parental strains				
		B	B1	C	D	D1
Parental strains	A	1/3	3/3	1/4	3/3	1/7
	A1	...	1/1	0/1	...	...
	B	...	2/2	2/4	...	3/10
	B1	...	...	1/2	2/2	...
	C	...	...	...	1/1	4/5

transmission is not randomly distributed in the table; nine of the ten crosses with B1 were aberrant, and all six of the crosses with D.

Since B1 and D are almost uniformly irregular in their behavior, they inflate the irregularities occurring in the other strains. If crosses to B1 and D are eliminated from consideration, the frequencies of irregularities with the other strains are considerably reduced. Strain A was involved in only three irregular crosses in a possible 13 (23 percent); A1 in 0/1 (0 percent); strain B in 6/17 (35 percent); strain C in 7/14 (50 percent) and strain D1 in 8/22 (36 percent). The frequency of irregular crosses in this selected sample is 12/34 or 35 percent.

The crosses listed in this series differed not only in the parental strains employed but also in the temperatures at which conjugation occurred. Unfortunately, the number of crosses at low or high temperatures was not large, but these suggest a temperature effect on genetic transmission. Again eliminating the B1 and D crosses, five crosses were carried out at 19°C and two (40 percent) were irregular; 21 were carried out at 26°C and five (24 percent) were irregular; eight were carried out at 32°C and four (50 percent) were irregular. In several cases crosses of identical parents at the same time gave regular results at one temperature and irregular results at another temperature. Although fragmentary, the data suggest that a greater hazard of irregularity exists at temperatures departing from the standard laboratory conditions under which the lines have been selected during the inbreeding process.

Other factors have also been examined to determine whether useful correlates of transmission irregularities could be identified. Both inbred series B1 and D have been refractory in recent inbreeding generations (Table 3). In spite of strenuous efforts in selecting vigorous lines, the frequency of viable pairs (Viable True Conjugants/Total Pairs) in crosses within these series continues to decline. In each generation two or three crosses are customarily made from separate pairs in the previous generation. Considerable variation is apparent among such crosses, but that cross which yields the highest frequency of viable progeny is selected for the next generation. This selective program has been adequate to maintain vigorous strains in some inbred series—A, B and D1 for example. It is apparently not adequate for other series, and once a severe depression is observed, the selection of a highly viable cross may be difficult even when many

TABLE 3

Maximum viability percentages (Viable Conjugants/Total Pairs) in crosses of inbred strains of variety 1*

Inbred series	Generation									
	5	6	7	8	9	10	11	12	13	14
A	67	48	93	87	83	97	59	81	100	..
A1	30	53	60	87	53	100	59	0	..	..
B	73	20	60	50	81	72	93	97	81	93
B1	90	87	33	68	13	5	4	0	..	..
C	80	35	43	13	77	70	83	52	31	58
D	43	97	79	43	80	60	25	40	31	17
D1	43	57	90	93	90	83	87	57	..	..
E	87	98	72	27	53	62	87	73	..	..
F	82	61	36	40	60	70	87	..	..	..

* Viable conjugants are defined as pairs which produce sufficient progeny within three to four days to be tested for non-conjugation, and which give negative mating tests. Some of the data, kindly supplied by S. L. ALLEN, have excluded pairs whose progeny show markedly depressed growth rates.

combinations of strains are employed. Moreover, the performance of strains in the inbreeding program is clearly related to their performance in outcrosses. Strains which produce less than 50 percent viable progeny upon inbreeding are also highly susceptible to aberrations in genetic transmission upon outbreeding.

The viability in outcrosses is a related index of aberration, though some strains which perform very poorly when inbred yield high frequencies of viable progeny when outcrossed. The median viability in normal crosses was slightly above 50 percent and the range was from 30 percent to 100 percent. The abnormal crosses in contrast had a median viability of less than 30 percent and a range from 10 percent to 87 percent. Although the viabilities in the two sets of crosses are clearly distinct, sufficient overlap in the distributions is apparent to render diagnosis on this basis a risky procedure.

Another criterion of aberration is "non-conjugation" (Nonconjugant Pairs/Viable Pairs). The normal crosses had a median frequency of nonconjugants of 25 percent and a range of 0-68 percent. The abnormal crosses had a median frequency of close to 50 percent and a range from 4-89 percent. Hence, again the ranges overlap and no cross can be positively diagnosed on the basis of non-conjugation, even though in general it may be stated that crosses with low viability and high frequencies of non-conjugation are much more likely also to manifest irregular genetic transmission.

DISCUSSION

The data presented here, though collected for another purpose and not followed up with the breeding analyses required for a thorough elucidation, strongly indicate that irregular genetic transmission occurs in crosses of many kinds among the inbred strains of variety 1, *T. pyriformis*. Indeed, over half of the 48 crosses examined provided some evidence of irregularity; nearly all the

crosses to inbred strains B1 and D yielded aberrant results. The aberrations detected were of two sorts; either one of the parents contributed nothing to most of the offspring, or some pairs received contributions from only one parent while other pairs received contributions from the other parent. In either case, however, a complete nuclear reorganization is indicated by the fact that the pairs analyzed gave rise to sexually immature progeny.

These observations raise several questions. The first is the mechanism responsible for the aberrant results. So far as the evidence goes, all the anomalies can be explained in terms of ALLEN's (1963) "genomic exclusion"; in individual pairs the micronuclear products of one parent may be lost and replaced by a product of the other parent. On the other hand, one cannot with the available data exclude cytogamy with unilateral death, induced selfing, or some other basis for the irregularities. A correlated breeding and cytological analysis of a series of crosses is now in progress and should provide definitive answers.

A second question is the bearing of these observations on the previously published studies on Tetrahymena. While the possibility of occasional aberrant pairs in these crosses cannot be discounted, any large scale failure of cytogenetic processes would have produced detectable peculiarities and these are not found in the published data. The several studies on intraclonal variation are largely unaffected, since the parental contributions are apparent or—in some cases—irrelevant. Peculiarities in genetic transmission appear to be a recent development.

The last and most important question is the prognosis for Tetrahymena genetics. The genetic irregularities certainly reflect cytological misbehavior, and this in turn is very likely a consequence of the inbreeding program. In the early stages of inbreeding (NANNEY 1957) several signs of inbreeding depression were described, but after five or six generations the strains appeared to be vigorous and normal. Since that time selection has been relaxed, but now some of the strains are again showing severe depression and all seem to be somewhat susceptible to cytogenetic irregularities. Homozygosis *per se* in a normally outbreeding organism (see SONNEBORN 1957) may result in an impairment of control mechanisms, but clearly some approximately homozygous combinations yield more regular behavior than others. The problem raised is that of maintaining reliable inbred strains and preventing further deterioration.

The major recommendation in this regard is that of practicing more rigorous selection. Instead of making two or three crosses in each generation and selecting the best for perpetuation, regardless of the absolute level of viability, efforts must be made in each generation to find clones capable of giving viable conjugants in a frequency of 80 percent or more. For inbred series such as B1 and D, the program will have to be reinstituted at earlier generations of inbreeding in hopes of recovering the germ plasm in useful form.

A second practice may also be useful in avoiding crosses which must later be rejected. The performance of different strains derived from the same parental cultures in the same cross is highly variable in some of the inbred series. These differences in performance are not ordinarily detected until the next generation

is produced. Perhaps a pre-testing of these latest derivatives in an inbred series would reduce the frequency of aberrant crosses. While time-consuming, this practice may help avoid the even more expensive employment of time in rejected crosses.

The efforts to "domesticate" Tetrahymena have, thus, not yet been completely successful. Great care must be exercised in maintaining strains and in monitoring crosses if the genetic results are to be valid.

SUMMARY

An analysis of 48 crosses conducted with inbred strains of variety 1 of *Tetrahymena pyriformis* reveals that aberrant genetic transmission occurred to some extent in over half (25). Two of the inbred series employed (B1 and D) were almost invariably irregular in their breeding behavior. Crosses manifesting irregularities were generally those in which the total viability was low and the frequency of nonconjugation was high. Indications of higher rates of anomalies in crosses above and below standard temperatures were available. Although these crosses were not ordinarily followed up by further breeding analyses, the F_1 data are suggestive of "genomic exclusion" as defined by ALLEN (1963); in any particular conjugating pair, the genetic contribution of one of the parents is lost. The high incidence of aberrations is believed to be a recent development and may reflect a failure of cytogenetic control systems in the highly inbred strains.

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