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Is There Selective Mating in *Tetrahymena* During Genomic Exclusion?[1]

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SYNOPSIS. Genomic exclusion is characterized by 2 rounds of mating. If exconjugants from different pairs remated at random after the first mating, we would expect a 1:2:1 ratio for genes present in heterozygous condition in the normal parent. An excess of homozygotes is observed which is similar for 2 different genes, suggesting that 10% of the rematings occur between exconjugants from the same Round 1 pair. Some but not all of these homozygotes can be attributed to a lack of separation of mates after the first round of mating. The rest may result because of differential mortality, induced autogamy or preferential remating.

UNIPARENTAL transmission of genes occurs in *Tetrahymena pyriformis* as a consequence of an abnormal form of conjugation called genomic exclusion. This unusual gene behavior is observed in the mating of a cell with a defective micronucleus and one with a normal micronucleus. Only the genes from the cell with the normal micronucleus appear in the progeny. If the normal cell is heterozygous, then more than one phenotypic class is observed.

The basis for uniparental transmission of genes resides in a series of unusual nuclear events which take place during conjugation (2). The normal mate contributes replicas of a single meiotic product to the diploid fertilization nucleus of each exconjugant, and when the exconjugants come apart the old macronucleus becomes the functional nucleus. The resulting exconjugants are heterocaryons, sexually mature and can remate immediately. When the exconjugants from the same pair remate, the progeny that appear are homozygous for all genes. If instead they remate with an exconjugant from a different pair, then the progeny can be heterozygous for some of the genes. For genes which are known to be heterozygous in the normal parent the 2 homozygous classes appear in a 1:1 ratio when all rematings involve exconjugants from the same pair. When remating of exconjugants from different pairs occurs, then the heterozygous class also appears. We would expect a 1:2:1 ratio of the 3 phenotypic classes if remating occurred at random.

Very early we observed departures from the expected 1:2:1 ratio for the H serotypes in such rematings and a deficiency of the heterozygous class (1). We also observed a deficiency of heterozygotes for the phosphatases when we examined *H* homozygotes. In this selected group of progeny the phosphatases seemed to be segregating in a 1:1:1 ratio. We did not, however, look at the distribution of phosphatases among *H* heterozygotes.

Does the distortion in the genetic ratio affect other genes or is it special to the *H* locus? The *H* character is a surface character and, since mating also involves a surface reaction, perhaps there is something peculiar about surface reactions which are manifested in the distorted ratio.

In this report we shall show that the same type of distortion occurs in the genetic ratios for the phosphatases, with an excess of homozygotes. How do these homozygotes arise during genomic exclusion? Is remating selective?

MATERIALS AND METHODS

Strains: C* is a semi-amicronucleate clone containing a mixture of cell types, some of which have a defective micronucleus and others which are amicronucleate (3). It was derived by inbreeding of the C strain (C-5573). In genotype it is H^E/H^E $P\text{-}1^B/P\text{-}1^B$.

AB clones have a normal diploid micronucleus and are derived from crosses of inbred strains A and B. In this paper we used 2 AB clones (AB-6 and AB-7a) which came from a cross of A-11613 and B-12614d. AB clones are heterozygous at both the *H* and *P-1* loci (H^A/H^D $P\text{-}1^A/P\text{-}1^B$).

Culture technics: Cultures of C* and AB were maintained at 23 C in bacterized medium (0.15% Cerophyl rye grass infusion inoculated with *Aerobacter aerogenes*). Previous to using them in crosses C* and AB-6 and 7a were cloned. Crosses (AB-6 × C* and AB-6 × AB-7a) were then set up with the subclones on a small scale and crosses yielding maximum viable progeny were then repeated on a large scale.

In preparing for a cross parental cultures were washed in Dryl's salt solution (6) and mixed. Mating begins after about 2 hr. Pairs were isolated at 4, 10, or 36 hr after the beginning of mating in different experiments. Single pairs were pipetted into different wells of depression slides containing bacterized medium and the exconjugants from the same pair were permitted to grow up together in the same well.

After 3-4 days the cultures were examined and classified into 3 types: Dead, mature and immature. *Dead* cultures included those which had died as well as those unable to undergo more than 2 or 3 fissions. *Mature* cultures contained pairs and reacted sexually to samples of a tester culture of non-parental mating

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TABLE 1. *Distribution of H serotypes among progeny of AB × C* cross isolated 36 hr after commencement of mating.*

Source	Ha	Had	Hd	Total
Table 7 (Allen '63)	34	60	43	137
Tables 6,7 (Allen '67)	29	36	39	104
Table 3 (Allen & Weremiuk '71)	57	94	60	211
Table 4 (Allen & Weremiuk '71)	135	195	133	463
Total	255	385	275	915
	.279	.421	.300	

Chi square (homogeneity) = 4.29, d.f. = 6, p = 0.5-0.7.
Chi square (1:2:1) = 23.5, d.f. = 2, p < 0.0001.

type in a "maturity test." Such cultures contain either Round 1 exconjugants or non-conjugants. *Immature* cultures did not contain pairs and were sexually unreactive in the maturity test. The *viability* of a cross is expressed as *the percentage of immature cultures.* Tests of H serotype and phosphatase-1 were carried out on samples of immature cultures.

Identification of H serotypes: The H serotypes were scored by mixing samples of bacterized cells grown at 23 C with each type of antiserum (anti-Ha, anti-Hd and anti-He). Immobilization of cells is specific and indicates the presence of a particular antigen (7). Homozygotes have only one of the antigens while heterozygotes have 2 of the antigens. The progeny of AB × AB and AB × C* crosses have either Ha, Had or Hd. These antigens are specified by genes from the AB parents.

Identification of phosphatase-1: Phosphatase-1 was scored on samples of cells rendered axenic by passage thru antibiotics and grown at 30 C in 1% proteose-peptone. Frozen-thawed extracts of concentrated cells were subjected to starch gel electrophoresis and the phosphatases visualized in the gels by incubation in an appropriate reaction mixture (4). Each genotype, $P\text{-}1^A/P\text{-}1^A$, $P\text{-}1^A/P\text{-}1^B$ and $P\text{-}1^B/P\text{-}1^B$, has a distinct and different phenotype which is easily scored by visual examination of the gels (5).

RESULTS AND DISCUSSION

1. *During genomic exclusion a significant departure from a 1:2:1 ratio occurs for the H serotypes.* Table 1 summarizes the published observations on the H serotypes appearing in the progeny of AB × C* crosses when pairs are isolated 36 hr after mating. For a random mating we expect 0.25 Ha + 0.50 Had + 0.25 Hd. We observe 0.279 Ha + 0.421 Had + 0.300 Hd. In all crosses there is a consistent deficiency of heterozygotes. When the data are pooled, the observed number of heterozygotes is very significantly different from the number expected in a 1:2:1 ratio (Chi square = 23.5, d.f. = 2, p < 0.0001).

2. *Significant departures from 1:2:1 ratio also occurs for the phosphatases.* Some years ago departures from a 1:2:1 ratio were observed for the phosphatases in *H* homozygotes (1). This of course was a selected group of progeny, and we could not answer the question of whether a distorted ratio would also be observed in an unselected group of progeny.

In the present series of experiments 2 sets of crosses were made: AB × C* and AB × AB. Pairs were isolated

TABLE 2. *Comparison of the distributions of H serotypes and phosphatases among progeny of AB × C* and (A × B)F₂ crosses.*

	AB × C*				
	P-1a	P-1ab	P-1b	Total	
Ha	22	26	28	76	.317
Had	21	51	25	97	.404
Hd	23	24	20	67	.279
	66	101	73	240	
	.275	.421	.304		

	(A × B)F_2				
	P-1a	P-1ab	P-1b	Total	
Ha	6	15	6	27	.237
Had	12	28	16	56	.491
Hd	7	16	8	31	.272
	25	59	30	114	
	.219	.518	.263		

Cross	Comparison	Chi square	d.f.	p
AB × C*	H vs. P-1	1.155	2	0.5-0.7
	H vs. 1:2:1	9.50	2	<0.01
	P-1 vs. 1:2:1	6.43	2	<0.05
(A × B)F_2	H vs. P-1	0.172	2	0.9
	H vs. 1:2:1	0.32	2	0.8-0.9
	P-1 vs. 1:2:1	0.58	2	0.7-0.8

36 hr after mating began and the immature progeny were scored for both their H serotypes and phosphatases. The results are shown in Table 2.

In the control cross (AB × AB) the distributions of the serotypes and phosphatases do not depart significantly from a 1:2:1 ratio, nor is there any trend toward an excess of *P-1* homozygotes among *H* homozygotes, or *H* homozygotes among *P-1* homozygotes.

A different story emerges when the data from the AB × C* cross are examined. There are significant departures from a 1:2:1 ratio for *both* the serotypes and the phosphatases (p < 0.01 and p < 0.05). The same degree of distortion is observed when the two sets of distributions are compared (p = 0.5-0.7) since there is a similar deficiency of heterozygotes (0.404 Had and 0.421 P-lab).

If we examine these data more closely we find that only *certain classes* show the distortion. Heterozygotes for *P-1* show a 1:2:1 ratio for the H serotypes and heterozygotes for *H* show a 1:2:1 ratio for the phosphatases. Neither of these distributions is significantly different from expected nor do they differ from each other. On the other hand, the homozygous classes depart significantly from a 1:2:1 ratio. Their distributions fit very closely a 1:1:1 ratio.

This point perhaps becomes clearer if we compare 2 different distributions for this 2-factor cross. With completely random mating we expect the following distribution (I):

1	2	1	4
2	4	2	8
1	2	1	4
4	8	4	

With non-random mating (preferential remating of homozygotes to the extent of 10%) we would expect (II):

1	1	1	3
1	2	1	4
1	1	1	3
3	4	3	

When we test the actual numbers observed against the distribution shown in II we find a close fit ($p = 0.5\text{-}0.7$).

These results suggest that departures from the ratios expected of random mating must be a general feature of genomic exclusion. An excess of homozygotes is observed for both types of character, not just the serotypes. It does not then seem to be gene specific but must arise thru some common biological mechanism.

From the observed numbers we can also obtain a measure of the degree to which the matings seem to depart from randomness. There is a deficiency of approximately 10% of the heterozygous class (30:40:30 instead of 25:50:25). This means then that there is a 10% excess of matings which lead to homozygotes.

3. *Some but not all of the homozygotes come from the lack of separation of mates during the 2 rounds of mating.* The simplest hypothesis which would account for the excess of homozygotes is that some of the pairs formed in the first round of mating do not separate before entering the 2nd act of conjugation. Such pairs would produce progeny completely homozygous for all genes. These progeny should also be immature.

Do we observe such unusual pairs? And how can we test our hypothesis?

We reasoned that if such pairs did indeed exist, we ought to detect them by observing immature progeny from pairs isolated at the beginning of the first round of mating.

What we did was to isolate a large number of pairs 4 and 10 hr after the beginning of mating. We examined the progeny for maturity and tested the immature progeny for their serotypes and phosphatases.

The results are shown in Table 3. We isolated 1,000 pairs at 4 hr and another 1,000 pairs at 10 hr. Out of the 4-hr isolates we observed 3 immature progeny and out of the 10-hr isolates we observed 42 immature progeny. All of these immature progeny were homozygotes. It appears then that some of the Round 1 pairs do enter Round 2 without separating. However, the frequencies observed are lower than expected on the basis of the distortions observed in the genetic ratios among 36-hr isolates.

The percentage of immature, homozygous progeny increased in the 10-hr isolates compared to those isolated at 4 hr. Ten hours is close to the time that separation of the Round 1 pairs normally occurs. It is thus possible that some of these progeny could have separated and preferentially remated. Even at 10 hr, however, the percentage of these progeny is about half that predicted: 4.2% compared to 10%.

TABLE 3. *Immature progeny from early isolations.*

	Distribution of Progeny				Phenotypic Distribution			
When Isolated	Dead	Mature	Immature	% Immature	HaP-1a	HaP-1b	HdP-1a	HdP-1b
4 hr	30	967	3	0.3	1	1	1	0
10 hr	53	905	42	4.2	9	10	8	15

The difference might be attributable to differential mortality with homozygotes superior in fitness compared to heterozygotes. If this were the case, we would expect to see an increasing number of deaths the later the time that pairs are isolated. However, no such trend is observed (see Table 6, 2).

Another explanation could also account for the increase in homozygotes. If in some of the matings of *unlike* Round 2 mates there was unilateral death and the survivor was induced to undergo autogamy, then the resulting progeny would be homozygous for all genes. Such progeny would be observed late; that is, only after both rounds of mating had occurred. They would not be found among the 4-hr isolates, but they might appear starting with the 10-hr isolates.

There are then several possible explanations for the distortion in the genetic ratios accompanying genomic exclusion. Some of the excess homozygotes can be attributed to a lack of separation of the exconjugants between the 2 matings. However, the frequency with which this occurs is not high enough to account for the observed excess. Barring differential mortality or induced autogamy, the only remaining hypothesis is that some of the Round 1 exconjugants must mate preferentially.

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