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Mating-type alleles in Illinois strains of *Tetrahymena pyriformis*, syngen 1

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1. INTRODUCTION

Mating-type potentialities in *Tetrahymena pyriformis*, syngen 1, are controlled by a single locus (*mt*) (Nanney, Caughey & Tefankjian, 1955). The *mt* allele present determines an array of possible mating types, but mating-type expression is determined by nuclear differentiation at the caryonide level (Nanney & Caughey, 1953; Nanney, 1956). Seven known mating types occur in this syngen, but only five of them (I–III, V, VI) can occur in strains of genotype mt^A/mt^A, and six of them (II–VII) in strains of genotype mt^B/mt^B. Among six wild strains previously screened for mating-type alleles, five were like mt^A, and one strain was a heterozygote for mt^B/mt^A (Nanney, 1959). Thus, of the twelve alleles present in these wild strains, only one was mt^B (I^-). Among strains with the *A*-like alleles (IV^-, VII^-), small differences were detected in the frequencies of the mating types produced, but they were qualitatively identical. This paper describes the mating-type alleles found in two new wild strains, obtained from Lake of the Woods, Mahomet, Illinois. The alleles present at certain antigenic and enzyme loci were also determined.

2. MATERIALS AND METHODS

The strains used in this study were *LW-A* and *LW-B* collected from two different lakes at Lake of the Woods, Mahomet, Illinois. Three representatives of the inbred families, family A (mt^A), family B 2 (mt^B) and family C 1 (mt^C) were also used. The derivation of these families and their allelic constitutions have been discussed in earlier papers (Nanney, 1959; Phillips, 1967*a*).

The methods used in crossing strains of *T. pyriformis*, syngen 1, and determining mating types, serotypes and isoenzyme types have been summarized elsewhere (Allen, 1960; Allen, Misch & Morrison, 1963*a*; Nanney & Caughey, 1953; Nanney & Dubert, 1960; Phillips, 1967*a*).

3. RESULTS

The phenotypes and genotypes of the two *LW* strains are given in Table 1. The genotypes were determined by a series of crosses. *LW-A* was successfully crossed with representatives of three different inbred families, but *LW-B* gave very few true conjugants when crossed with representatives of the inbred families. An *LW* inbred line strain was established by obtaining an F_1 from an *LW-A* × *LW-B* cross. Ten different F_1's were further inbred until several new inbred series were established.

The mating-type frequencies observed among the progeny of the crosses are given in Table 2. The frequencies in inbred strains *A* and *B* are included for comparison. The data show that *LW-A* has only (IV^-, VII^-) alleles, but that a (I^-) allele is present in *LW-B*. When *LW-A* was crossed to family A, homozygous for mt^A (IV^-, VII^-), no IV or VII mating types were found; thus the *LW-A* alleles cannot produce these types. When

LW-A was crossed to inbred family B2, homozygous for mt^B (I^-), all seven mating types were found. Therefore, *LW-A* has at least one allele allowing production of mating type I. Previous studies have shown that mt^A/mt^B heterozygotes have about one-half the number of IV's and VII's found in mt^B/mt^B homozygotes (Nanney, 1959). The frequencies of IV and VII in this cross were about half those for the mt^B homozygote; this result is consistent with a homozygous genotype for *LW-A*. All seven mating types were also obtained in the *LW-A* × *LW-B* F_1 cross; hence *LW-B* must contain at least one allele with the potentialities for IV and VII. In this cross the frequency of mating-type IV,

Table 1. *Genotypes and phenotypes of LW-A and LW-B*

Strain		Gene locus					
		H	*T*	*mt*	*E*-1	*E*-2	*P*-1
LW-A	Phenotype	D	A	III	B	C	B
	Genotype	*D/D*	*A/B*	*A/A*	*B/B*	*C/B*	*B/B*
LW-B	Phenotype	A	A	VI	B/C	B	A
	Genotype	*A/A*	*A/A*	*A/B*	*B/C*	*B/B*	*A/A*

Table 2. *Mating-type frequencies in crosses involving LW-A and LW-B*

Cross	Genotype	Mating-type frequencies							
		I	II	III	IV	V	VI	VII	Total
LW-A × *LW-B*	—	0·13	0·18	0·23	0·12	0·07	0·23	0·02	119
LW-A × *A*	mt^A/mt^A	0·18	0·40	0·02	0·00	0·06	0·31	0·00	93
LW-A × *B*2	—	0·07	0·22	0·02	0·37	0·04	0·20	0·08	102
LW-A × *LW-B* F_6	mt^B/mt^B	0·00	0·21	0·14	0·46	0·05	0·05	0·05	235*
A × *A*†	mt^A/mt^A	0·25	0·17	0·17	0·00	0·10	0·30	0·00	809
B × *B*†	mt^B/mt^B	0·00	0·15	0·09	0·47	0·05	0·14	0·10	1090
A × *B*†	mt^A/mt^B	0·12	0·14	0·15	0·24	0·07	0·18	0·12	399

* 0·04 selfers were obtained in this cross.
† Data from Nanney, 1959.

about one-fourth that found in the mt^B homozygote, suggests that *LW-B* may be heterozygous for the mt^B allele. This was confirmed in the F_2 by the appearance of many (IV^-, VII^-) clones. One F_2 clone which had mating types IV and VII as well as I was further inbred eventually through the F_6; its mating-type frequencies are shown in Table 2. This F_6 inbred strain is very similar to mt^B/mt^B homozygotes, for it lacks mating-type I and has a high frequency of mating-type IV. Thus, *LW-A* appears to be homozygous for a (IV^-, VII^-) allele and *LW-B* appears to be a heterozygote.

The strains were also characterized for alleles at the other known loci in syngen 1. The H serotypes are ciliary immobilization antigens expressed at 20–35 °C (Margolin, Loefer & Owen, 1959; Nanney & Dubert, 1960). In mature heterozygotes only one allele is ordinarily manifested (Nanney, 1964; Nanney, Nagel & Touchberry, 1964) and breeding analyses are required to determine the full genotype. *LW-A* was Hd in phenotype and *LW-B* was Ha. All F_1 synclones were initially Had, as would be expected for a cross between two homozygotes (Table 1).

A second series of ciliary antigens, T, is expressed above 38 °C, and phenotypic differentiation also occurs in lineages heterozygous at this locus (Phillips, 1967*a*, *b*). Both *LW-A* and *LW-B* were Ta in phenotype (Table 1). However, the cross of *LW-A* × *LW-B*

produced eleven Ta synclones and thirteen Tab synclones; one of the parental strains may be heterozygous for the T^B allele. The cross of *LW-A* to strain *B*2 (T^C/T^C) produced both Tac and Tbc progeny, demonstrating that *LW-A* has the T^B allele and must be the heterozygote.

Three genetic loci controlling enzyme specificities have been analysed by Allen (1960, 1961), and Allen, Misch & Morrison (1963*b*), using starch-gel electrophoresis with specific enzyme localization. The *E*-1 and *E*-2 loci control esterase isoenzymes of different substrate specificities. The *P*-1 locus controls an acid phosphatase. Phenotypic differentiation is also found for heterozygotes at these loci (Allen, 1965), and again breeding analyses are required for full genotypic determination. The isoenzyme phenotypes are given in Table 1. In the *LW-A* × *LW-B* F_1 there were five E-1B/E-1C, seven E-1B/E-1B. This would be expected if *LW-B* were an $E\text{-}1^B/E\text{-}1^C$ heterozygote and *LW-A* were an $E\text{-}1^B/E\text{-}1^B$ homozygote. For the *E*-2 enzymes, five E-2B/E-2C : six E-2B/E-2B were obtained in this cross. Thus, *LW-A* is heterozygous for $E\text{-}2^B/E\text{-}2^C$ and *LW-B* is an $E\text{-}2^B/E\text{-}2^B$ homozygote. The *P*-1 enzymes in the *LW-A* × *LW-B* F_1 all had the hybrid P-1A/P-1B band and a cross of *LW-A* × C1 ($P\text{-}1^B/P\text{-}1^B$) yielded all P-1B progeny. Thus, *LW-A* is a $P\text{-}1^B/P\text{-}1^B$ homozygote and *LW-B* is a $P\text{-}1^A/P\text{-}1^A$ homozygote.

4. DISCUSSION

These results are significant in several respects. The fact that only two cells (four genomes) were studied from a limited area, but allelic diversity was found at all six of the loci examined, supports the interpretation that syngen 1 is a highly polymorphic form, an outbreeder, and likely to be obtained in nature heterozygous for many genes (see Sonneborn, 1957; Nanney, 1957; Orias, 1960). Interestingly enough, although only two alleles were found at each of the loci examined, these appear identical to previously identified alleles. This suggests that the polymorphism is limited in the sense that relatively few genic forms exist for each locus. The data for the mating-type locus are especially significant. Originally many alleles were anticipated at this possibly complex locus, each one determining a different array of possible mating types (Nanney, 1959). However, a survey of eight cells (sixteen genomes) has revealed only two qualitatively different alleles, both occurring in widely separated geographic areas. Thus the (IV$^-$, VII$^-$) and (I$^-$) alleles appear to be basic structural alternatives at this locus.

Finally, the failure to obtain any new mating types provides additional evidence against the hypothesis of paired complementary mating-type substances (Metz, 1954; Sonneborn, 1957), at least for *Tetrahymena*. This theory was proposed because the number of mating types within a syngen in *Paramecium aurelia*, *P. caudatum*, *P. bursaria* and *P. multimicronucleatum* corresponds to a geometrical progression. Initial studies on the number of mating types in the syngens of *Tetrahymena* failed to demonstrate such a progression, but the possibility that further collections would yield the 'missing' mating types could not be disregarded (Nanney, 1959). However, this possibility seems less likely in view of the results described in this paper.

SUMMARY

Two new wild strains of *T. pyriformis*, syngen 1, have been examined for new mating-type, serotype and isoenzyme alleles. No new alleles at the mating type (*mt*) locus were found, but the two alleles previously found were extracted. No new alleles were found at the other five loci examined, but two alleles were obtained for each locus. These observations indicate that a limited polymorphism exists for these loci in natural populations of *T. pyriformis*, syngen 1.

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