Theor Appl Genet (1994) 89: 838–846

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Genetics of *Ustilago violacea*. XXXII. Genetic evidence for transposable elements

Received: 21 March 1994 / Accepted: 29 April 1994

Abstract Crosses between *Ustilago violacea* mutant strains with different color phenotypes that were derived from the 1.A1 and 2.A2 laboratory strains yielded, as expected, bisectored teliospore colonies with the parental colors as well as the a-1 and the a-2 mating-types. Generally, wild teliospore collections usually produced sporidia of both mating-types, providing two-mating-type (TMT) strains. Occasionally, however, sporidia with only one mating-type allele, a-1 or a-2, were obtained from teliospores, providing one-mating-type (OMT) strains. Crosses between OMT and laboratory strains with different color phenotypes gave (1) bisectored teliospore colonies with the parental colors or colonies with a parental color and a nonparental color and (2) nonsectored colonies with the nonparental color or with the parental color. The frequencies for the occurrence of non-parental color ranged from 41% to 93%, depending on the strain. The yield of teliospore colonies was usually reduced for these crosses. In many of these teliospore colonies, morphologically-altered sporidia (MAS phenotype) were observed. The morphology and the size of the sporidia with the MAS phenotype differed from those of teliospore colonies of the crosses between the laboratory strains. In addition, these sporidia did not form conjugants. A cross involving the TMT strains C449 yielded the MAS phenotype as well as a high incidence of tetrad colonies with a nonparental color. The high degree of instability of the parental color phenotypes, and the high frequency of the appearance of nonparental color phenotypes as well as the appearance of the MAS phenotype, are in accord with the presence of active and inactive transposable elements in the OMT strains, TMT strains, and laboratory strains.

Key words Transposable elements · Teliospore colony
One-mating-type strain · Color phenotype
Locus instability

Communicated by L. Alföldi

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Introduction

Ustilago violacea (Pers.) Roussel is a bipolar, heterothallic (a-1, a-2) heterobasidiomycete, pathogenic for susceptible species in the Caryophyllaceae (Fischer and Holton 1957). The smutted anthers of diseased plants contain purple, uninucleated diploid teliospores that are disseminated by insect vectors or wind. On an appropriate substrate, the teliospore germinates, produces a short tube, the promycelium, into which the nucleus migrates and undergoes meiosis to yield four haploid nuclei. After meiosis, a linear tetrad of two a-1 and two a-2 basidiospores are formed on the promycelium (Fischer and Holton 1957). The mating-type locus is centromere-linked (Saltiel and Garber 1984).

Each basidiospore of a tetrad buds to produce a sporidium, which multiplies by budding to form a yeast-like teliospore colony. Each of the four sporidial clones in the teliospore colony has the genotype of the progenitor basidiospore. Mature, solitary, uninucleated sporidia in the teliospore colony have slightly tapered ends and display little variation in dimensions and morphology during the first 7–10 days after teliospore germination (Fischer and Holton 1957). Numerous conjugants involving haploid a-1 and a-2 sporidia are observed when sporidia from the teliospore colonies are mixed on water agar and incubated overnight at 14–18°C (Day and Jones 1968).

Day and Jones (1968) selected one a-1 and one a-2 sporidium from a large pink colony formed by a teliospore harvested from a smutted anther of *Silene alba* to initiate the laboratory strains 1.A1 a-1 and 2.A2 a-2. These two laboratory strains, which require thiamine, have been maintained in culture and storage for more than 20 years and have been stable for the color phenotype, thiamine-deficiency, and mating-type allele. Strains 1.A1 and 2.A2, and

all of the different color, nutritional and morphological mutants obtained by UV and chemical mutagenesis, have been used for extensive transmission genetic studies in this species (Garber and Day 1985; Day and Garber 1988).

Teliospores collected from plants with smutted anthers in the field and from smutted anthers of herbarium specimens gave, in addition to pink colonies, yellow, pumpkin, white, and orange colonies, or bisectored colonies with one of the possible combinations of these color phenotypes (Garber et al. 1978). Haploid strains with one of the color phenotypes from each of the 32 wild collections have also remained stable for almost 20 years, excepting for an occasional color mutant.

In extensive genetic analyses of the color phenotypes, Garber et al. (1975, 1980) found that the orange (*o*), pumpkin (*p*), yellow (*y*) and white (*w*) sporidial colony colors were determined by closely linked (< 1 cM), centromere-linked, recessive mutant genes that form the following linkage map: *o-p-y*-centr-*w*. The pink phenotype is dominant (*o*+ *p*+ *y*+ *w*+) and results from the overproduction of cytochrome c; the orange, pumpkin and yellow phenotypes result from different combinations and concentrations of carotenes; the mutant white strains and white strains from the collections have either no detectable or very-low concentrations of the highly-unsaturated carotenes and a reduced concentration of cytochrome c (Will et al. 1982, 1984; Ruddat and Garber 1983).

Crosses between different color phenotypes usually produced bisectored teliospores colonies with the parental color. Only occasionally were nonsectored colonies, with one or the other parental color, detected (Cattrall et al. 1978). Faster growth of sporidia with one of the color phenotypes may have masked the other parental color. Colonies with nonparental colors, however, sometimes occurred with an unexpected frequency. Some teliospore colonies were trisectored, displaying the parental colors and pink as the nonparental color (7–17%). More frequent were nonsectored colonies with the nonparental color pink (21–62%). Sporidia from each sector of the trisectored colonies were haploid and stable with respect to their color phenotype. The nonparental pink colonies contained both large oval and typically haploid sporidia (Cattrall et al. 1978, Fig. 1). As the pink colonies increased in diameter, they exhibited the parental color sectors (*y* and *w*), which had haploid sporidia and were stable with respect to their color phenotype. The pink sectors had both large oval and haploid sporidia and sporidial transfers gave pink (+), *y*, and *w* colonies. The trisectored colonies resulted from crossing-over between the *w* locus and the centromere and gave a tetrad of *y* +, *y w*,+*w*, and ++ (pink) haploid basidiospores. The pink teliospore colonies resulted from the production of two viable disomic (n+1) basidiospores with *y*+ and +*w* chromosomes and two inviable n-1 basidiospores in a tetrad. The *y* and *w* sectors in the pink colonies originated from disomic sporidia that lost one or the other chromosome to yield haploid sporidia during their vegetative multiplication (Cattrall et al. 1978).

The fine structure map of the complex white (*w*) locus was constructed, based on the recombination values from crosses between strains with UV-induced *w* mutantions in the 1.A1 and 2.A2 laboratory strains (Garber 1980). Complementation tests indicated the presence of 2–3 cistrons in tandem (Garber 1980). However, crosses between the *w* mutants in the 1.A1, 2.A2 laboratory strains and *w* strains in the wild collections, unexpectedly, did not place the *w* mutations in the wild collections to sites in the *w* locus or to the cistrons in this complex locus (Garber 1982).

Garber and Owens (1980) examined numerous teliospore colonies from intra- and inter-collection crosses of different wild collections of white and yellow strains for pink sectors and for disomic (*w*/*w* and *y*/*y*) pink colonies. Sixteen of twenty-seven intercollection crosses gave only white teliospore colonies and the remaining nine crosses produced relatively-few bisectored (*w*/+, *w*/*y*, and *w*/*p*) colonies, nonsectored yellow colonies and pink disomic colonies. Certain intracollection crosses among the yellow strains, however, gave a significantly large percentage, approximately 86%, of bisectored or nonsectored colonies with the nonparental white phenotype. In seven of the ten crosses, strain 2.C503 *y* was the common parent. Collection C503 was one of eight wild collections represented by sporidia with only one-mating-type (OMT strain).

The observation that strain 2.C503 *y*, represented by sporidia with only one mating-type allele, was involved in crosses that yielded unexpectedly-high frequencies of teliospore colonies with a *nonparental* color phenotype led us to examine crosses involving OMT strains from other collections for teliospore colonies with a nonparental color phenotype. This report presents the results of crosses involving strains from collections represented by sporidia with one mating-type allele (OMT strains), strains from collections with a-1 and a-2 sporidia (TMT strains), and mutant strains derived from the laboratory strains. The data were analyzed with respect to (1) the yield of teliospore colonies, (2) the morphology of sporidia in teliospore colonies, (3) the presence or absence of conjugants for sporidia in the teliospore colonies and (4) the color phenotype or phenotypes of teliospore colonies.

Materials and methods

Strains

Collections of *U. violacea* (Pers.) Roussel, aka *Microbotryum violaceum* (Pers.: Pers.) Deml & Oberw., made and maintained at the University of Chicago were identified by the letter "C" before the identification number. Collections represented by sporidia with only one mating-type allele were termed one-mating-type (OMT) collections and strains from such collections were identified by an asterisk after the identification number. Collections represented by sporidia with one or the other mating-type allele were termed two-mating-type (TMT) collections. Strains from each collection have either a "1" or "2" preceding the collection number to identify their mating-type allele and a symbol following the indentification number to indicate their colony color. For example, 1.C426* *w* is an a-1 strain from an OMT collection at the University of Chicago and has white sporidial colonies. The pink (+) OMT strain 1.JA* a-1 was kindly furnished by Dr. J. Antonovics, Duke University.

Sectors of colonies initiated from a single sporidium in teliospore colonies from smutted anthers in the 32 field and herbarium

specimens were scored for color and mating-type alleles. Eight collections had only one mating-type allele, either a-1 or a-2 (OMT strains), and the remaining collections had both mating-type alleles (TMT strains) (Garber et al. 1978). From collections with bisectored teliospore colonies, a-1 and a-2 sporidia with pink, white, yellow, pumpkin or orange color phenotype were selected to initiate strains.

The pink strains 1.A1 a-1 and 2.A2 a-2 were kindly furnished by Dr. Alan W. Day, University of Western Ontario. These and the UV-induced mutant strains, including a-1 *y his-1* and 2.14.14 a-2 *w pdx* derived from them, were termed laboratory strains. These strains are therefore TMT strains.

The TMT strains 1.C449 a-1 *y* and 2.C449 a-2 *w* were selected for an intracollection cross because, unlike other strains in the collections, strain 2.C449 a-2 *w* had sporidia with typically haploid dimensions and morphology but also some sporidial chains. Sporidial colonies of strain 2.C449 a-2 *w* had a typically round shape but produced projections of elongated sporidia or sporidial chains at the periphery as the colonies increased in diameter. Sporidial transfers gave colonies in which these projections persisted.

Culture medium

The formulation of the minimal and complex media and the culture conditions were the same as in Day and Jones (1968) and Garber et al. (1975). Conjugants were detected by mixing sporidia from a single teliospore colony on water agar and incubating overnight at 18°C. Procedures for inoculating seedlings of *S. alba* at the rosette stage, harvesting smutted anthers from large, unopened buds, and initiating teliospore colonies, were those of Day and Jones (1968).

The teliospores in a suspension were counted with a hemocytometer. Aggregates of 2–10 teliospores were often present in the samples and were counted as a single teliospore. Pipetting the teliospore suspension to make dilutions usually dispersed teliospore aggregates, thereby increasing the observed number of teliospore colonies above the expected number. By the same token, an observed number of teliospore colonies less than the expected number was an underestimate of the number of viable teliospores.

Teliospores from each cross were incubated on complex medium and the teliospores and microcolonies were examined daily by microscopy over a period of 5 days for germination, sporidial multiplication, and colony morphology.

Haploid segregants were obtained from diploid sporidia, using p-fluorophenylalanine as the haploidizing agent (Day and Jones 1969).

Results

Yield of teliospore colonies

Three of four crosses between OMT strains, two of six crosses between OMT and laboratory strains, and one of two crosses between TMT and laboratory strains, had reduced yields of teliospore colonies (Table 1). It was not possible, however, to relate reduced yields with a specific OMT or TMT strain.

Table 1 Yield of teliospore colonies, number of teliospore colonies with 2n sporidia, and number of teliospore colonies with the MAS phenotype

Cross	Genotypes	Number of teliospore colonies[a]				No. colonies examined	MAS[c] phenotype
		Yield					
		Estimated	Observed	%	2n[b]		
Intercollection cross							
		OMT* × OMT* strains[d]					
8	1.JA*[e] *y* × 2.C448* +	168	130	77	1	40	29
9	1.C435* + × 2.C448* +	240	320	>100	–	24	0
10	1.C439* y^l × 2.C448* +	312	147	47	12	40	18
11	1.C426* *w* × 2.C448* +	–	–	–	–	20	18
12	1.C432* *w* × 2.C448* +	1200	984	82	–	24	0
		OMT* × laboratory strains (TMT)					
1	1.JA* *y* × 2.14.14 *w*	240	191	80	4	15	4
3	1.C435* + × 2.14.14 *w*	130	146	>100	–	56	39
4	1.C426* *w* × 2.14.14 *w*	280	124	80	1	20	20
5	1.C432* *w* × 2.14.14 *w*	195	427	>100	–	24	0
6	1.C439* y^l × 2.14.14 *w*	–	–	–	3	16	4
13	1.A1 *w* × 2.C448* +	240	283	>100	–	20	12
22	1.C2 *w* × 2.C448* *w*	255	274	>100	–	20	0
		TMT × laboratory strains (TMT))					
23	1.C430 *w* × 2.A2 +	80	120	>100	–	29	12
24	1.C449 *y* × 2.A2 +	280	124	44	–	16	0
Intracollection cross							
25	1.C449 *y* × 2.C449 *w*	85	122	>100	–	–	–

[a] (–) indicates not determined

[b] Teliospore colonies with a pink phenotype and diploid sporidia in approximately 7×10^2 colonies

[c] MAS phenotype, morphologically-altered sporidia: size and morphology of sporidia different from wild-type

[d] OMT* (one-mating-type strains), from wild collections represented by strain(s) with only one mating-type allele, identified by an asterisk following the strain number; TMT (two-mating-type strains) from wild collections represented by strains with the a-1 or a-2 mating-type allele; laboratory strains – 1.A1 and 2.A2 strains and mutants derived from either strain

[e] +, pink; $+^l$, light pink; *w*, white; *y*, yellow; y^l, light yellow

Five crosses involving OMT strains gave 1–12 (0.7–8.1%) pink teliospore colonies with diploid sporidia (Table 1). Many crosses, approximately 1000, between laboratory strains did not, however, yield teliospore colonies with diploid sporidia (unpublished data). Teliospores from crosses 8, 10 and 12 (Table 1) either did not produce detectable microcolonies or produced microcolonies with only a few nonbudding sporidia.

Sporidial morphology and the morphological-altered sporidia (MAS) phenotype

Sporidia of teliospore colonies from crosses with laboratory strains are normally uniformly solitary and elliptical, 29–30 μm long and 13–14 μm at the largest diameter. Sporidia of many teliospore colonies from 9 out of 14 crosses involving OMT strains and intracollection cross 25 were heterogeneous with respect to dimensions and morphology included: (1) small to large, solitary sporidia with slightly tapered ends but indistinguishable from haploid sporidia of TMT strains, (2) large, round, vacuolated and solitary sporidia, (3) solitary sporidia with a pair of terminal buds, (4) large, oval and solitary sporidia and (5) chains of 2–5 swollen sporidia. The relative frequencies of each type were not determined. This array of different types of sporidia was termed the morphological-altered sporidial (MAS) phenotype (Table 1). Teliospore colonies from crosses with OMT strains contained mostly (1) small to large solitary sporidia, (2) large oval and solitary sporidia, (3) few or none of the other types of sporidia. This was termed the moderate MAS phenotype. The frequencies of teliospore colonies with the MAS phenotype may have been underestimated because all of five types of sporidia had to be present to be assigned to the MAS phenotype.

Teliospore colonies from crosses between laboratory strains had neither the MAS nor the moderate MAS phenotype. The solitary sporidia in the teliospore colonies after 7–10 days of incubation had only one terminal bud and there was little or no heterogeneity in size or morphology.

Conjugants for sporidia in teliospore colonies

Sporidia of teliospore colonies from crosses between laboratory strains gave 60–80% conjugants. Conjugants also formed between diploid and haploid sporidia. The latter type of conjugants are readily detected by their noticeably-large diploid and small haploid sporidia.

In the teliospore colonies from crosses involving OMT and TMT strains conjugations occurred usually between two small sporidia but occasionally between one large and one small sporidium. The frequencies of teliospores yielding conjugants depended on the detection of conjugants and not on the relative numbers of conjugants (Table 2). The frequencies of teliospore colonies yielding conjugants from crosses 12 and 24 were 92% and 88%, respectively, and for the remaining crosses, 0% to 42% (Table 2).

Table 2 Frequency of teliospore colonies with conjugants in intercollection crosses[a]

Cross	Genotype[b]	Total number of teliospore colonies	Conjugants[c]
		OMT* × OMT* strains	
8	1.JA* *y* × 2.C448* +	40	9
9	1.C435* + × 2.C448* +	24	8
10	1.C439* y^l × 2.C448* +	40	13
11	1.C426* *w* × 2.C448* +	20	6
12	1.C432* *w* × 2.C448* +	24	22
		OMT* × laboratory strains (TMT)	
1	1.JA* *y* × 2.14.14 *w*	15	3
3	1.C435* + × 2.14.14 *w*	56	11
4	1.C426* *w* × 2.14.14 *w*	20	3
5	1.C432* *w* × 2.14.14 *w*	24	10
6	1.C439* y^l × 2.14.14 *w*	16	3
13	1.A1 *w* × 2.C448* +	20	0
22	1.C2 *w* × 2.C448* *w*	20	5
		TMT × laboratory strains (TMT)	
23	1.C430 *w* × 2.A2 +	29	3
24	1.C449 *y* × 2.A2 +	16	14

[a] OMT*, wild collections represented by strain(s) with only one mating-type allele; TMT, wild Collections with a-1 and a-2 strains; laboratory strains, strains 1.A1 and 2.A. and mutants derived from these strains

[b] +, pink; $+^l$, light pink; *w*, white; *y*, yellow; y^l, light yellow

[c] Conjugants involved only a large and a small (n) sporidium or two small (n) sporidia

Color phenotypes in bisectored and nonsectored teliospore colonies

An aliquot sample of teliospores from each cross provided teliospore colonies to determine the color phenotypes. Crosses involving OMT strains gave teliospore colonies with a nonparental color, except certain crosses involving strains 1.JA*, 2.C448* and 2.503*. Nonparental colors were not observed in teliospore colonies from crosses involving TMT and laboratory strains. However, the intracollection cross between the TMT strains 1.C449×2.C449 gave more than 50% nonparental color, while the cross between 1.C449 and the laboratory strain 2.A2 produced no nonparental color (Table 3).

Of the seven crosses yielding teliospore colonies with a nonparental color, only cross 3 gave teliospore colonies with a parental color but with different nonparental colors (Table 3). In four crosses (9, 11, 12, 3) the parental colors, pink (+) or pink (+) and white (*w*), changed from pink to the nonparental color yellow (y). The reverse, a change from yellow to pink was observed in cross 20. In cross 5 the white parental color was replaced with the nonparental color yellow (*y*); in cross 4 the color change was from the parental color white (*w*) to a nonparental light yellow (y^l). Either parental color, pink (+) or white (*w*), was replaced by yellow in different teliospore colonies from cross 3.

Bisectored colonies from crosses 9, 12, 20 and 25 had sectors with a parental and a nonparental color; the bisectored teliospore colonies from 3, 5, and 11 had either sec-

Table 3 Teliospore colony color phenotypes in intercollection and intracollection crosses

Cross	Genotype[a]	Teliospore colonies				
		Phenotypes[b] Color and number			Total number	% Nonparental color
Intercollection crosses[c]						
		OMT* × OMT* strains				
8	1.JA* *y* × 2.C448* +	*y*/+(39)	**y**(50)	+(41)	130	0
9	1.C435* + × 2.C448* +	**y**/+(166)	**y**(80)	+(74)	320	77
10	1.C439* y^l × 2.C448* +	**y**/+(34)	**y**/+(48)	+(65)	147	0
11	1.C426* *w* × 2.C448* +	**y**/*w*(22)	**y**/+(8)	*w*/+(4)		
		y(44)	*w*(49)	+(52)	179	41
12	1.C432* *w* × 2.C448* +	**y**/*w*(967)	**y**(4)	*w*(13)	984	99
20	1.C417* *y* × 2.C503* **y**	*y*/+(541)	+(2)	*y*(5)	548	99
		OMT* × laboratory strains (TMT)				
1	1.JA* *y* × 2.14.14 *w*	*y*/*w*(89)	*y*(89)	*w*(65)	191	0
3	1.C435* + × 2.14.14 *w*	**y**/+(20)	**y**/*w*(22)	*w*/+(4)		
		y(21)	+(49)	*w*(29)		
		y/*w*/+(1)			146	44
4	1.C426* *w* × 2.14.14 *w*	**y^l**(48)	*w*(73)	+(2) [2N]	123	39
5	1.C432* *w* × 2.14.14 *w*	**y**/*w*(388)	**y**(10)	*w*(29)	427	93
13	1.A1 + × 2.C448* +	$+^l$/*w*(3)	$+^l$(123)	*w*(158)	283	0
21	1.A1 + × 2.C503* *y*	*y*/+(541)	*y*(5)	+(2)	548	0
22	1.C2 *w* × 2.C448* +	*y*/+(124)	*y*(85)	+(65)	274	0
		TMT × laboratory strains (TMT)				
23	1.C430 *w* × 2.A2 +	*w*/+(29)	*w*(56)	+(35)	120	0
24	1.C449 *y* × 2.A2 +	*y*/+(107)	*y*(9)	+(8)	124	0
Intracollection cross						
		TMT × TMT strains				
25	1.C449 *y* × 2.C449 *w*	+/*w*(32)	+(37)	*w*(53)		
		+/$+^l$			123	57

[a] Genotypes: +, pink; $+^l$, light pink; *w*, white; *y*, yellow; y^l, light yellow. Symbols in bold type indicate a nonparental phenotype

[b] Nonparental color phenotype in bold letter

[c] OMT*, strains from wild collections represented by sporidia with one-mating-type allele identified by an asterisk following the strain number. TMT, strains from wild collections with a-1 and a-2 sporidia. Laboratory strains: strains 1.A1 and 2.A2 and mutants derived from pink strains 1.A1 a-1 and 2.A1 a-2

tors with a parental and a nonparental color or each parental color. Cross 4 gave nonsectored teliospore colonies with either a parental or a nonparental color. Teliospore colonies from cross 10 with one light-yellow (y^l) parent had a bright-yellow (*y*) phenotype; teliospore colonies from cross 13 with one pink (+) parent had a light-pink ($+^l$) color.

The frequencies of bisectored or nonsectored teliospore colonies with a nonparental color ranged from 41% to 99%. Crosses 5, 9 and 12 with the highest frequencies of nonparental color had no tested teliospore colonies with the MAS phenotype, indicating that the teliospore colonies had mostly solitary small-to-large sporidia comparable to typically-haploid sporidia in dimensions and morphology (Table 2).

One teliospore colony from cross 3 was trisectored with *y*/*w*/+ sectors. The dimensions and morphology of the sporidia in each sector were typical for haploid sporidia.

Color phenotypes and MAS Pphenotype in intracollection cross 25

Teliospores from intracollection cross 25 (1.C449 *y*2.C449 *w*) produced +/*w* and $+^l$/+ bisectored colonies and *w* and + nonsectored colonies (Table 3). Four bisectored teliospore +/*w* colonies and one bisectored +/$+^l$ teliospore colony provided sporidia from each sector to test for MAS phenotype. In each of these teliospore colonies, sporidia from one sector had an obvious MAS phenotype and sporidia from the others a moderate MAS.

Sporidia from the sectors with the MAS phenotype in the four bisectored colonies (*w*/+) did not yield conjugants, while sporidia from the other four sectors with a moderate MAS phenotype gave conjugants. Sporidia in both sectors from the fifth bisectored colony (+/+) did not form conjugants.

In 10 of 15 nonsectored colonies no conjugants were observed. These sporidia had the MAS phenotype and also did not form conjugants when tested against haploid a-1 and a-2 sporidia from the laboratory strains. The sporidia of the remaining five nonsectored colonies with a moderate MAS phenotype formed conjugants. The lack of conjugants for sporidia from colonies or sectors with the MAS phenotype may be an additional trait of this phenotype.

Sporidia from bisectored and nonsectored colonies from the teliospores of the different collections had been trans-

ferred several times prior to determining their mating-type allele and possible nutritional requirements. In cross 25, however, sporidia from sectors of teliospore colonies with the MAS phenotype that had no detectable conjugants formed conjugants after two transfers the of sporidia. The frequencies of the conjugants were not determined. Sporidia from 14 nonsectored colonies with the MAS phenotype, including ten colonies without detectable conjugants, were twice transferred and then tested for conjugation. Ten colonies yielded conjugants; one colony had only haploid a-1 sporidia that conjugated in a test using a-2 sporidia; and three colonies did not yield detectable conjugants.

Diploid strains from crosses 1 and 6

Diploid sporidia of pink teliospore colonies from cross 1 (1.JA* *y*×2.14.14 *w*) and from cross 6 (1.C439* y^l×2.14.14 *w*) yielded diploid sporidia that were used for haploidization. Diploid strain 1 gave the following 13 haploid segregants: *w* a-1 (3), *w* a-2 (6), *y* a-1 (2) and *y* a-2 (2). Diploid strain 6 gave the following haploid segregants, which were not tested for mating-type allele: *y* (9), y^l (2) and *w* (13). While the segregants did not show a nonparental color, the *y* segregants obviously differed in color intensity compared with the y^l parental strain.

Discussion

We have analyzed three unstable color phenotypes in *U. violacea*: yellow (*y*), white (*w*) and pink (*y*+ *w*+). Their mutability is restricted to diploid cells committed to meiosis: preteliospores (Garber et al. 1984) and teliospores, but not to diploid sporidia. The three phenotypes are stable in the haploid parental strains. Highly-mutable loci furnish acceptable indicators for transposable elements, provided that other possible sources can be eliminated, e. g., disomy involving the chromosomes with heterozygous genes (*y* +/+ *w*) in a haploid species, ring chromosomes with dominant alleles in disomic cells with heterozygous genes or in diploid cells or in trisomic cells with the recessive alleles, or a mutator gene(s).

Transposable elements: teliospore colonies with a nonparental color phenotype and the MAS phenotype

The activity of transposable elements offers a plausible explanation for the high frequencies of teliospore colonies with a nonparental color, for the morphologically-altered sporidia (MAS phenotype) and for the apparent stability of the color phenotypes and the absence of the MAS phenotype in teliospore colonies from crosses involving certain OMT, TMT and laboratory strains. Unstable loci, usually involving a nutritional deficiency or ascospore color, indicate the activity of transposable elements. Unstable loci have been demonstrated in different fungal species, such as *Saccharomyces cerevisiae* (Greer et al. 1980), *Ascobolus immersus* (Decaris er al. 1980; Nicolas et al. 1987), *Coprinus radiatus* (Ozier-Kalogeropoulos and Guerdoux 1975), and *Verticillium dahliae* (Puhalla 1980). Transposable elements are proposed to account for the genetic observations in all of these species except *V. dahliae*. Transposable elements have been identified in yeast (Farabaugh and Fink 1980) and also in filamentous fungi, *Fusarium oxysporum* (Dabousssi et al. 1992) *Magnaporthe grisea* (Hamer et al. 1989; Dobinson et al. 1993), and a strain of *Neurospora crassa* from the Ivory Coast (Kinsey and Helber 1989). Laboratory strains of *N. crassa*, however, appear to be free of active transposons since they possess a mechanism that destroys certain types of repeated DNA sequences (Selker 1991).

We postulate two types of transposable elements for *U. violacea*: (1) nonautonomous transposable elements (TE) that are defined by their loci and are responsible for the color phenotypes and (2) controlling elements (CE) that are responsible for the transposition of TE and which are defined by their role in the transposition of the TE as well as the MAS phenotype and their loci. A TE-C in the *y*+ locus (pink phenotype) produces the yellow phenotype and is termed a TE-C*y*; a TE-C in the *w*+ locus (pink phenotype) produces the white phenotype and is termed TE-C*w*. A TE-C in *y* inactivates that locus and results in a nonparental color, white or pink. No TE-C in either locus results in the pink (*y*+ *w*+) phenotype and is termed TE-C+.

In diploid cells a trans-acting controlling element, CE, mediates the transposition of TE-C and of another CE. The transposed CE inserted into a meiosis gene, termed CE-MAS, can account for the MAS phenotype. An inactive (cryptic) controlling element (CE-CO) accounts for the stability of the laboratory and tested TMT strains. It does not mediate transposition of TE or CE. The CE-CO accounts for the absence of a nonparental color and of the MAS phenotype in the teliospore colonies from crosses between the laboratory strains. The results of cross 3 (1.C435*+×2.14.14. *w*), which gave teliospore colonies with an unexpected nonparental color, can be explained by assuming that the CE-CO element in the laboratory strain 2.14.14.*w* was transposed by UV-treatment into the *w*+ locus and thus acquired a TE-C*w* element. This element was then transposed by the CE element into the other parental strain. The nonparental yellow (*y*) in teliospore colonies from cross 3 with one parental color and two nonparental colors may have resulted from a transposition because the parental strain 1.C435* + has a trans-acting CE.

The *y*/*w* bisectored teliospore colonies from cross 3, however, may have resulted from a spontaneous mutation in the *y*+ locus of this parental strain, resulting in a change from pink to yellow (+ → *y*) in one haploid nucleus of the dikaryotic infectious hyphae (Garber and Owens 1980). Clonal multiplication of this mutant nucleus would yield teliospore colonies with the unexpected combination of parental and nonparental colors.

The transposition of CE into a meiosis gene simulated mutability for this locus and resulted in the MAS phenotype. The difference between the MAS and the moderate

MAS phenotypes, and presumably the intergrades between these extremes from teliospore colony to teliospore colony from the same or different crosses, may reflect the site of insertion of the CE proximal to, or in, the meiosis gene. The transposition of a CE from an active site or from the site of the meiosis gene into a third site yielded an inactive CE-CO element. This element provides the basis for the absence of a nonparental color or the MAS phenotype in teliospore colonies from crosses between laboratory strains.

The CE may be present in different sites in sporidia from different OMT and TMT strains. For example, teliospore colonies from cross 1 between the OMT strain 1.JA* and a laboratory OMT strain gave teliospore colonies with the MAS phenotype but a nonparental color was not observed. Strain 1.JA* presumably had the CE-MAS and the other parental strain did not have a CE but may have had a cryptic CE. The laboratory strains presumably had a CE-CO and crosses between these strains did not yield teliospore colonies with either the MAS phenotype or with a nonparental color.

In assigning the different types of TE and CE to the OMT, TMT and laboratory strains (Table 4), we considered the following criteria: (1) the occurrence of a nonparental color and (2) the MAS phenotype in teliospore colonies from intercollection and intracollection crosses. Laboratory strains 2.14.14 *w* and 1.C2 *y* were assigned a CE-CO because neither gave a nonparental color nor the MAS phenotype in their teliospore colonies when crossed within laboratory strains. Crosses between OMT strains, which were assigned a CE-C, and the laboratory strain 2.14.14 *w*, however, gave teliospore colonies with a nonparental color. This observation was responsible for proposing the UV-induced transposition of a TE-CO element in the pink laboratory strain 2.A2 into the *w*+ locus yielding 2.14.14 *w*. The UV-induced *y* mutation in 2.A2 was presumably genic in origin.

Crosses 1 and 8, which had strain 1.JA* *y* as a common parent and strains 2.C448* + and 2.14.14 *w* as the other parent, did not yield teliospore colonies with a nonparental color but with the MAS phenotype. This observation was responsible for assigning a CE-MAS to strains 2.C448* + and 1.JA* *y*. Teliospore colonies from cross 22 (1.C2 *y* × 2.C448* +) had the parental colors and the MAS phenotype.

Crosses between OMT strains assigned a CE element and a laboratory strain with TE-C*w* and CE-CO elements have teliospore colonies with a nonparental color and the MAS phenotype. These observations required the CE to mediate the transposition of both the TE-C*w* and CE-CO in strain 2.14.14 *w*. There is no evidence for CE mediating the transposition of the CE-MAS. The results of crosses involving meiotic segregants from crosses between certain OMT strains and a laboratory strain might show that the MAS phenotype is determined by a mutant gene and not a CE-MAS.

Alternative models that account for the coincidence of teliospore colonies with a nonparental color and the MAS phenotypes need to consider that both involve transposable elements. The different sites of the TE and CE and the effect of an element in each site provided sufficient flexibility to explain the nonparental color and the MAS phenotype only from certain intercollection crosses involving the OMT strains. They also explained the presence of the MAS phenotype and the absence of the nonparental color phenotypes as well as the absence of both the nonparental color and the MAS phenotype in crosses involving laboratory strains.

Table 4 Transposable and controlling elements in tested strains of *U. violacea*

Strain[a]	Transposable element[b]			Controlling element[c]		
	TE-Cw	TE-Cy	TE-CO	CE	CE-MAS	CE-CO
1.C417* *y*		×		×		
1.C426* *w*	×			×		
1.C430 *w*	×				×	
1.C432* *w*	×			×		
1.C435* +			×	×		
1.C439* y^l		×		×		
1.C449 *y*		×		×		
1JA* *y*		×			×	
2.C448* +			×		×	
2.C449 *w*	×			×		
2.C503* *y*		×		×		
2.14.14 *w*	×					×
2.A2 +			×			×
1.C2 *y*			×			×

[a] +, pink; *y*, yellow; *w*, white
[b] TE-Cw, transposable element in *w*+ locus; TE-Cy, transposable element in *y*+ locus; TE-CO, transposable element in neither *w*+ nor *y*+ locus.
[c] CE, trans-active controlling element; CE-MAS, controlling element in a locus for normal meiosis gene; CE-O, inactive (cryptic) controlling element.
* Indicates one-mating-type strain (OMT*)

Aneuploidy: teliospore colonies with a nonparental color and the MAS phenotype

As an alternative to transposable elements, disomy (*y* +/+*w*) in the basidiospores of the tetrads of *U. violacea* may provide a chromosomal source for the three unstable color phenotypes, yellow (*y*), white (*w*) and pink (*y*+ *w*+). The nonparental pink-colored teliospore colonies from crosses between certain UV-induced *w* strains or between a *y* and a *w* strain were disomic for chromosomes with complementing color mutations (Cattrall et al. 1978). They also showed the MAS phenotype and contained haploid sporidia in the sectors with the parental *y* and *w* colors. The nonparental pink-colored sector in trisectored colonies resulted from crossing-over between the *w* mutations or between the *w* locus and the centromere (Cattrall et al. 1978).

The relatively-high frequency of teliospore colonies with a nonparental color from certain intercollection crosses with OMT and TMT strains and intracollection cross 25 (TMT strains) may also be accounted for by disomy. Assuming that meiotic nondisjunction yielded the

sporidia for these strains, the sporidia would be stable disomics for the chromosomes with the closely-linked color mutations. For example, the genotype of disomic pink strains could be *y* +/*w* + so that a cross involving such a strain might yield a nonparental *y* or *w* sector in a teliospore colony or a nonsectored colony. A stable disomic strain, however, would have the MAS phenotype and would produce *y* and *w* sectors with haploid sporidia.

Crosses between a white (*w*) and a pink (*y*+ *w*+) strain occasionally (≪1%) yielded trisectored teliospore colonies with *y*, *w* and pink (*y*+ *w*+) sectors that had haploid sporidia (Cattrall et al. 1978). It is unlikely, however, that crossing-over between the *y* and *w* loci would yield the high frequencies of teliospore colonies with a nonparental color.

Meiotic nondisjunction: the MAS phenotype

Meiotic nondisjunction during germination of the teliospores may be a plausible explanation for the MAS phenotype in teliospore colonies from crosses involving certain OMT and TMT strains. Teliospores producing at least one viable euploid or aneuploid basidiospore would form a teliospore colony, which may have 1–4 sporidial clones, depending on the number of viable basidiospores in the tetrad.

Viable aneuploid basidiospores can produce clones of aneuploid sporidia with different numbers and combinations of extra chromosomes in each sporidial clone from the progenitor basidiospore. The different types of morphologically-altered sporidia may be phenotypic expressions of different combinations and numbers of extra chromosomes. The absence of conjugating sporidia from the teliospore colonies with the MAS phenotype could be an additional expression of aneuploidy. The appearance of conjugants after at least two sporidial transfers, indicating haploid a-1 and a-2 sporidia, would result from the loss of one of the disomic chromosomes with the a-1 or a-2 mating-type allele. Sporidial transfers have yielded haploid sporidia with only one mating-type allele, indicating that the aneuploid progenitor basidiospore had only the one chromosome with a mating-type allele.

A plausible explanation for the reduced yields of teliospore colonies from certain intercollection crosses and not from others, for colonies with diploid sporidia, and for the absence of detectable conjugants by using chromosomal repatterning or the genetic constitution of the teliospores from intercollection crosses, is not intuitively obvious. Meiotic nondisjunction could, however, be responsible for the different components of the MAS phenotype. A mutable gene(s) responsible for normal meiosis in the diploid, germinating teliospores would account for the MAS phenotype. Proposing several mutant genes among the collections assumes that these genes are likely to be mutable and retained in the different collections. There may be merit, therefore, to consider transposable elements also in causing the MAS phenotype. Molecular information should provide the basis for a definitive interpretation.

Genetic and molecular evidence for unstable loci in fungal species

In a study of genetic instability involving ascospore color in the heterothallic ascomycete *A. immersus*, a cross between two wild strains from stock 50 with wild-type brown ascospores gave an ascus with four wild-type and four white ascospores (Decaris et al. 1980). The genetic analysis of unstable revertants from the white ascospores revealed a complex pattern of genetic instability involving different loci for ascospore color and timing at different stages of the life-cycle of the species. At least one-third of the spontaneous ascospore color mutants from a revertant×revertant cross were unstable and mapped at 14 loci in at least seven chromosomes.

The genetic analysis of revertant instability for genes *b*1, *b*2, *b*3, *b*5 and *b*7 indicated that reversion modalities were not gene-specific but rather mutant or site-specific. An unstable strain did not revert as a male parent when the female was noninducing; the same unstable mutant reverted when the female was inducing. Comparable complex relationships were also found in the genetic analysis of other revertant strains. All of these observations were explained by proposing transposable elements with "relatively definite integration sites" comparable to the bacterial insertion sequence elements.

Some molecular evidence supports transposable elements as being responsible for genetic instability in *A. immersus* (Decaris et al. 1980). The DNA from mycelial nuclei of all of the 13 unstable strains from stock 50 appeared as a linear molecule of 4×10^6 Da (Type A); the other 14 stable or revertant strains from the same stock lacked this DNA molecule. The DNA of the two stable and three revertant strains differed in their molecular mass. None of the nine strains from the other stocks had low molecular-weight DNA. The presence or absence of the proposed transposable elements in the unstable or stable strains is indicative but not definitive. The extraction procedure may have been responsible for the presence of the low-molecular-mass DNA.

Mitotically-unstable *HIS4C* strains in *S. cerevisiae* yielded spontaneous *HIS4C*-segregants with frequencies of 1–10% by two different mechanisms: (1) precise excision of the *HIS4C*-inserted transposable element restored *HIS4C* function and (2) insertion of the element caused loss of the entire chromosome (Greer at al. 1980). The latter mechanism was detected by using aneuploid strains heterozygous for the *HIS4C*+ inserted element and a multimarked chromosome XII to give the following genotype: *HIS4C*+ *ASP*+ *RAD*+ *GAL*+/*asp- rad- gal-*. In addition, *HIS4C*- segregants frequently occurred during meiosis for a *HIS4C*+ insertion diploid strain. Transposition moved the *HIS4C* locus from chromosome III to chromosome XII at a site close to the centromere. The absence of a deletion in chromosome III implied replication of the locus in the transposition process. Strains that have transposed the HIS4 locus concomitantly became mutagenic, producing chromosomal rearrangements and auxotrophic mutations. In the R2 transposition strain, the originally centromere-

linked *HIS4C* gene was no longer centromere-linked but remained in the same chromosome. Molecular evidence indicated two types of transposable elements: (1) an element in the *HIS4C* structural gene capable of transposition, excision and mutator activity and (2) an element spontaneously inserted into the *HIS4A* regulatory region controlling expression of the *HIS4* structural gene.

The switching of mating-type alleles (the cassette model) in homothallic strains of *S. cerevisiae* and *Schizosaccharomyces pombe* are familiar examples of the role of transposable elements in changing a specific phenotype with specific targets for transposition. In these examples, the genetic evidence is supported by molecular evidence (Klar 1989). The Ty transposable elements in *S. cerevisiae* have been implicated in altered phenotypes (Farabaugh and Fink 1980).

One aspect of the cassette model for the switching of mating-type alleles in *S. cerevisiae* may have ramifications for the model proposed for the switching of colony-color phenotypes in *U. violacea*. In the cassette model, the silent MAT cassettes flank the active MAT (mating-type) locus in the following order in chromosome III: HML(a)-centromere-MAT-HML(a) (Klar 1989). In the switching of mating-type alleles, a copy of one silent cassette with the information for the other mating-type allele replaces the resident mating-type in the MAT locus to change the mating-type allele. This process is generally repeated after each mitotic division. In this example there is one target locus for each of the two cassettes and transposition from HML(a) to the Mat locus is pericentric. In *U. violacea*, the transposition of the proposed element responsible for the new colony color phenotype is pericentric and may involve two sites in each chromosome arm.

Patterns of genetic instability and stability associated with transposable elements have been related to specific developmental stages during the life-cycle of the species when transposition presumably occurs. These patterns not only differ among species but also within a species, as in *S. cerevisiae* and *A. immersus*. The intraspecific differences may involve members of different families of transposable elements, as in maize and *Drosophila melanogaster* (Berg and Howe 1989). As more fungal species receive attention from geneticists, it is likely that mutable loci will be found to harbor transposable elements.

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