MUTATIONS AFFECTING SEXUAL CONJUGATION AND RELATED
PROCESSES IN *SACCHAROMYCES CEREVISIAE*. II. GENETIC
ANALYSIS OF NONMATING MUTANTS^{1,2,3}

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ABSTRACT

Rare diploids formed by sterile mutants have been studied by tetrad analysis. Sixteen classes of mutants representing at least five distinct genetic loci have been defined. One group of mutations, isolated only in α , maps at the mating-type locus, while none of the others shows any linkage to mating type. Some of the mutations are nonspecific for mating type, while others act only on α or α . In addition, mutations were found that prevent sporulation when heterozygous in diploids. These appear to be mutations of the mating-type alleles.

IN the yeast *Saccharomyces cerevisiae*, sexual conjugation between two cells is a function of the alleles at the mating-type locus, which is also involved in the control of other cellular processes, including sporulation and meiosis, X-ray survival, and UV-induced mitotic recombination. To study these processes and their genetic control, we have isolated haploid mutants that could not mate with cells of the opposite mating type. The isolation and phenotypic characteristics of these mutants is described in the preceding paper (MACKAY and MANNEY 1974). In this paper we report the results of genetic analysis of the rare diploids formed between these mutants and normal strains of the opposite mating type. We also report recombination and linkage studies between several of the mutants that could be crossed to each other.

MATERIALS AND METHODS

Yeast strains, sterile mutants that were isolated, systems of nomenclature, and media have been described in the preceding paper (MACKAY and MANNEY 1974).

Additional media include the following:

PET: Glycerol, 3%; dextrose, 0.25 g/l; yeast extract, 10 g/l; Bacto-Peptone, 10 g/l; agar,

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20 g/l. PET contains a nonfermentable carbon source and was used to score the respiratory-deficient (petite) phenotype.

BTB gal: Yeast extract, 10 g/l; Bacto-Peptone, 20 g/l; galactose, 20 g/l; brom thymol blue (1% in ethanol), 33 mg/l; pH adjusted to 8 with N/2 NaOH (HAWTHORNE and MORTIMER 1960). BTB gal is a fermentation medium used for scoring high-frequency mating (complementation).

Incubation conditions and methods for sex factor assays, mating, and sporulation have also been described in the previous paper.

Tetrad analysis: After sporulation, the asci obtained were dissected by the procedure of JOHNSTON and MORTIMER (1959) using Glusulase (Endo Laboratories) to digest the ascus walls. With some diploids, no four-spored asci were apparent, so three-spored asci were dissected.

The ability of each spore strain to mate and its mating type were scored by two separate complementation systems; one method detects only high-frequency mating (as between two wild-type strains) and the other high- and low-frequency mating (as between a sterile mutant and a test strain). The two methods are described below:

Method 1: Detection of high-frequency mating. All of the mutants and most of the test strains carry the recessive allele *gal2* that prevents the utilization of galactose. The *gal2*-bearing spore cultures were cross-streaked on BTB gal with α and α *gal1* testers. If a high-frequency (wild-type) mating occurred, the indicator in the medium changed from blue-green to yellow within 24 hours. Low-frequency (rare) mating between a spore culture and a tester did not yield enough diploids to cause the color change in one day.

Method 2: Detection of low-frequency mating. Each spore was cross-streaked on YEPD with α and α testers carrying nutritional markers complementary to those in the spores. After overnight incubation, the YEPD mating plates were replica-plated to a medium (usually MV) selective for the diploid formed from a spore culture and a tester. High-frequency mating resulted in confluent growth in the cross-streaked area, low-frequency mating gave papillations in the cross-streaked area, while lack of mating yielded no growth.

Random spore analysis: Diploids were analyzed by the random spore method of GILMORE (1967). After sporulation, approximately 10^7 cells were treated with Glusulase for 1–2 hours and then suspended in water. The suspension was sonicated until the spores had been separated and then diluted for plating on YEPD to give approximately 100 colonies per plate. After 2–3 days' incubation, the YEPD master plate was replica-plated to one MV plate to identify any prototrophic diploid clones and to a fresh YEPD lawn of a test strain with the appropriate mating type and markers. The YEPD mating plate was incubated for 24 hours and then replica-plated to MV. If mating occurred between a spore clone and the test strain, the resulting diploids could grow on MV, while the spore clone itself could not.

For some diploids, a simple qualitative random spore method was used (suggested by R. K. MORTIMER). After the asci had been treated with Glusulase, a loopful of the digestion mixture was streaked on YEPD to obtain single colonies. The plates were stamped to MV and to a YEPD lawn of a tester and then treated as above. Again, if mating occurred between a spore clone and the test strain, the resulting diploids could grow on MV. With this method, however, the percentage of sporulation and the number of spore clones was not determined.

RESULTS

For each of the sterile mutants that mated at low frequency with cells of the opposite mating type, two or three diploid clones were dissected for tetrad analysis. Some of the sterile mutations failed to recombine with mating type, and are apparently mutations of the mating-type locus or are very closely linked to this locus. Others, however, showed no linkage to mating type. The sterile mutations segregating in these crosses could be characterized as either specific or nonspecific for mating type. The former eliminate mating ability in only one mating type, and the latter prevent mating in both mating types. For example, if a diploid is

heterozygous for an α -specific *ste* mutation, the phenotypes of parental ditype (PD) asci would be 2 α :0 α :2 *ste*, of nonparental ditype (NPD) asci 2 α :2 α :0 *ste* and of tetratype (T) asci 2 α :1 α :1 *ste*. One α spore in T asci and both α spores in NPD asci should carry the mutation but still mate normally as α cells. If the defect is nonspecific, the phenotypes are 2 α :0 α :2 *ste* (PD), 0 α :2 α :2 *ste* (NPD) and 1 α :1 α :2 *ste* (T).

The tetrad data for these heterozygous diploids were analyzed for specificity of the *ste* mutations and their linkage to the mating-type locus. Phenotypic properties of each mutant, such as sex factor production, low-frequency mating, and sporulation of the LFM diploids, were determined as described in the previous paper. With these results, the sterile α mutants can be grouped into eight distinct classes and the sterile a mutants into six classes. A summary of these is presented in Table 1, with typical segregation data given in Table 2. The classes and the results supporting them are discussed individually below, with reference to these tables.

As discussed in the preceding paper, none of the sterile a mutants respond to α factor, and only one α mutant responds to a factor. In addition, all mutants in the first eight classes mate with wild-type cells at low frequency, forming diploids that can sporulate. The individual classes are further characterized by the following specific properties:

Class 1: Derived from α parent strains, these ten mutants carry *ste* defects that are closely linked to or at the mating-type locus (*MT*). Typical tetrad data, such

TABLE 1

Class	Mating type of parent strain	Sex factor production	Low frequency mating	Sporulation of LFM diploids	Specificity of <i>ste</i>	Linkage to MT	Number of mutants
0	<u>a</u>	<u>a</u> -factor ⁻	+	+	unknown	close	0
1	<u>α</u>	<u>α</u> -factor ⁻	+	+	unknown	close	10
2	<u>α</u>	<u>α</u> -factor ⁻	+	+	unknown	close	1*
3	<u>a</u>	<u>a</u> -factor ⁺	+	+	<u>a</u>	none	2
4	<u>α</u>	<u>α</u> -factor ⁺	+	+	<u>α</u>	none	5
5	<u>a</u>	<u>a</u> -factor ⁻	+	+	<u>a</u> or <u>α</u>	none	24
6	<u>α</u>	<u>α</u> -factor ⁻	+	+	<u>a</u> or <u>α</u>	none	16
7	<u>a</u>	<u>a</u> -factor ⁺	+	+	<u>a</u> or <u>α</u>	none	2
8	<u>α</u>	<u>α</u> -factor ⁺	+	+	<u>a</u> or <u>α</u>	none	16
9	<u>a</u>	<u>a</u> -factor ⁻	+	-			7
10	<u>α</u>	<u>α</u> -factor ⁻	+	-			6
11	<u>a</u>	<u>a</u> -factor ⁺	-				3
12	<u>α</u>	<u>α</u> -factor ⁺	-				32
13	<u>a</u>	<u>a</u> -factor ⁻	-				23
14	<u>α</u>	<u>α</u> -factor ⁻	-				7
TOTAL							154

* This α mutant responds to a -factor.

TABLE 2

Segregation data from crosses of representative ste mutants by wild-type strains

Class	Mutant	Original mating type	<i>ste</i> vs. mating type		
			PD	T	NPD
1	VC2	α	42	0	0
2	VN33	α	35	0	0
3	VAB2	$\underline{a}$	1	13	4
4	VC3	α	6	30	4
5	VV3	$\underline{a}$	7	30	4
6	VE2	α	9	45	12
7	VAI2	$\underline{a}$	3	12	1
8	VJ3	α	10	37	7

as that for VC2 (Table 2), demonstrated the absence of recombination between *MT* and *ste*, since only PD asci were obtained. For VC2, the upper limit for the map distance between *ste* and *MT* is 1.2 centimorgans. The other mutants in this class also yield only PD asci, with upper limits for the length of the *MT-ste* interval of 0.94 to 5.0 centimorgans. The sterile mutations may be at the mating-type locus itself or at another gene closely linked to *MT*. At present, therefore, the mutations have been designated *ste1* with the reservation that they may represent mutations at *MT*, at a closely linked gene, or both.

None of these α mutants or α *ste* spores derived from them produced α -factor. Therefore, the sex factor properties of the mutants are a result of the mutation, since they segregated with *ste* in the spores.

Class 2: One mutant, VN33, was unique among the sterile α strains in that it responded to *a* factor, although the response was less pronounced than in wild-type α cells. Otherwise, VN33 had the same properties as the class 1 α mutants; it failed to produce α factor and its *ste* mutation did not recombine with *MT*. All 35 asci dissected were PD asci, so the upper limit for the map distance between *ste* and *MT* is 1.4 centimorgans. For these reasons, it was classified as an *ste1* mutant and given the gene symbol *ste1-5*. Sterile α spores derived from VN33 also responded to *a* factor and were unable to produce α factor.

Although mutations at *MT* or *ste1* represent a major group of α mutants, no mutants of this type have been found among the sterile *a* strains. Such a mutational site may not exist in *a* cells or may confer a very different phenotype in *a* mutants than in α mutants. The *a ste1* mutants may be among the *a* strains that do not mate with α cells even at low frequency. In any case, there exists a mutational difference between *a* and α at the mating-type locus that must be explained by models for the structure of this locus.

Class 3: These two mutants were isolated from *a* parents and carry mutations that are *a*-specific and unlinked to the mating-type locus. The lesions can be carried in the genome of α cells without affecting the mating ability or other

phenotypic properties of the strain. Tetrad analysis for VAB2 (Table 2) yielded 1 PD:4 NPD:13 T. The ratio observed and the ascus phenotypes could result from two different situations: the segregations of an unlinked *a* specific *ste* or the segregation of an unlinked suppressor with *ste* closely linked to MT as in the *ste1* mutants. In the former case, *ste* can be recovered from 1/2 of the α spores in T asci and both of the α spores of NPD asci. In the latter situation, *ste* is carried only in *a* spores (i.e., *a ste sup*). The segregation in the diploid from VAB2 was shown to be the result of an unlinked *a*-specific *ste* by crossing an α spore from a NPD ascus with a wild type *a* and recovering the *a*-specific *ste* mutation among the spore progeny. The other *a* mutant in this class has the same properties as VAB2.

Both of these mutants and *a ste* spores derived from them produce *a* factor. The α spores that carried *ste* in their genome were able to make α factor, respond to *a* factor, and mate as wild-type α cells with *a* strains.

Class 4: Five α mutants have α -specific *ste* mutations that are unlinked to the mating-type locus, and are therefore analogous to Class 3 mutants. A typical mutant is VC3, which gave 6 PD:4 NPD:30 T, when crossed with a normal mating strain. The α -specificity of *ste* in VC3 was demonstrated by crossing both *a* spores of T asci with wild type α cells. If *ste* is α -specific and unlinked to MT, one *a* of each pair would carry *ste*. For five such pairs, one *a* of each ascus always contained an α -specific *ste* in its genome.

All five of the class 4 mutants and αste spores derived from them were capable of making α factor; the *ste* mutation had no apparent effect on *a* cells, since *a ste* spores produced *a* factor, responded to α factor, and mated as wild-type *a* cells.

Class 5: These strains carry mutations that are nonspecific for mating type, and are the largest class of nonmating *a* mutants. None of these show linkage to the mating type locus, with typical tetrad data such as that for VV3 in Table 2 (7 PD:4 NPD:30 T). Two of the mutants have suppressible defects, one of which can be suppressed by a suppressor that also suppresses two other alleles, *lys1-1* and *can1*. It has been established that *lys1-1* is an ochre (UAA) mutation (HAWTHORNE 1969; GILMORE, STEWART and SHERMAN 1971), so presumably *ste* and this *can1* allele, of spontaneous origin in this strain, are also ochre mutations.

None of the 24 *a ste* mutants or *a ste* spores derived from them produce *a* factor. Furthermore, in αste spores derived from these mutants, *ste* prevents the production of α factor and the response to *a* factor.

Class 6: The 16 sterile α strains in this class all have *ste* mutations that are nonspecific for mating type and unlinked to the mating-type locus. For the representative mutant, VE2, tetrad analysis yielded 9 PD:12 NPD:45 T (Table 2). All of the mutants and αste spores derived from them failed to make α factor; similarly, *a ste* spores were unable to produce *a* factor or respond to α factor. Therefore, these mutants are analogous to the class 5 *a ste* mutants.

Class 7: Like the class 5 *a* mutants, these two strains have nonspecific mutations that do not show linkage to the mating-type locus. Tetrad data for VAI2 are

3 PD:1 NPD:12 T (Table 2). Unlike the class 5 strains, however, both mutants produce detectable amounts of *a* factor.

None of the *a ste* spores from VAI2 produced detectable quantities of *a* factor. The *a ste* spores from VAI2 were similarly unable to produce *a* factor or respond to *a* factor. The significance of these results is uncertain, however, since the mutant itself is a weak producer of *a* factor, often giving negative results on assays.

Class 8: This class consists of 16 α -factor-positive mutants with nonspecific *ste* mutations unlinked to the mating-type locus. Typical tetrad results are those for VJ3, 10 PD:37 T:7 NPD (Table 2). All of the mutants produced α factor, as did *a ste* spores derived from them. None of the *a ste* spores from these made *a* factor, although the insensitive *a* factor assay may not have detected reduced quantities of the factor.

Classes 9 and 10: The sterile *a* and α mutants that mate with wild-type strains to form LFM diploids incapable of sporulating were described in the preceding paper.

Classes 11-14: Sterile mutants that do not mate even at low frequency were discussed in the preceding paper.

Although the mutants can be placed in classes, the number of genes involved cannot be determined from the above results. All of the phenotypes associated with the *ste* mutations unlinked to the mating-type locus could be manifestations of mutations in a single gene. Since classical complementation and allelism tests are not possible with these mutations, recombination (or lack of it) between two mutations was used to estimate the minimum number of loci represented by these mutants.

Recombinational analysis: For these studies of sterile mutations, the diploids must be *a/a steX/+ +/steY*, where the *steX* and *steY* loci may be linked or unlinked to each other. Such diploids have been isolated for certain combinations of mutants. (1) One parent carries a sterile mutation specific for the other mating type; e.g., *a steX* by *a steY* where *steX* is α -specific. (2) Both parents are temperature-sensitive sterile mutants; diploids have not been recovered from *a steX* by *a steY* matings when both are nonspecific and only one is temperature-sensitive. Because of these restrictions, only a limited number of recombination tests have been possible. Frequent recombination between two mutations indicates that they are not at the same genetic locus. If no recombinant spores are recovered in the spore sample, the two mutations are linked but are not necessarily in the same gene.

The mutations that are closely linked to the mating type locus were designated *ste1* (classes 1 and 2, Table 1). The other *ste* mutations, which all fail to show linkage to *MT*, represent one or more additional genes. Therefore, the *ste* mutation in VAB2 was arbitrarily chosen as *ste2*; this mutation is a α -specific and unlinked to *MT* (class 3). Any mutation which segregates independently from *ste2* must be in a different gene.

Cross A: *a steX* (α -specific) by *a ste2* (α -specific). The *ste2* mutation was obtained in an α spore which mates as a wild-type α , since *ste2* is α -specific. The

TABLE 3

a steX (α -specific) $\times$ α *ste2* (α -specific)

Spore	Ascus phenotype			Total asci
	I	II	III	
A	<i>ste</i>	<u>a</u>	<u>a</u>	
B	<i>ste</i>	<i>ste</i>	<u>a</u>	
C	<u>α</u>	<u>α</u>	<u>α</u>	
D	<u>α</u>	<u>α</u>	<u>α</u>	
Observed	11	0	0	11
Expected if no recombination	11	0	0	11
Expected if unlinked	6	5	0.3	11.3

presence of *ste2* in the α spore was confirmed by crossing it to a wild-type *a* and recovering sterile *a* spores in the tetrads. The α *ste2* strain was mated at low frequency with the other class 3 *a* mutant; the cross and the tetrad data are summarized in Table 3. Since no recombinant asci were recovered, the two mutations cannot be assumed to be in different genes. The observed distribution of ascus types is significantly different ($P < 0.025$) from the expected distribution for two unlinked mutations.

Because of the α -specificity of the mutations, for a short map interval, 1/2 of the recombinant asci should have the same phenotype as the type I asci (Table 3) and 1/2 the type II phenotype. Therefore, only 1/2 of the total recombinant asci will be detected as recombinants without further crosses and analysis. Thus, the upper limit for the map distance between *ste2* and *steX* equals the highest possible frequency of recombinant asci times 100 or, if the next ascus tested were recombinant, $(1/12) \times 100 = 8.3$ centimorgans.

Cross B: *a steX* (α -specific) by α *ste2* (α -specific). Since both strains carried mutations specific for the other mating-type, they could mate normally. The *a steX* strain was derived from VQ3, one of the class 4 sterile mutants with α -specific mutations unlinked to mating type, while α *ste2* was made as in cross A above. The presence of these mutations in the two strains was established by outcrossing them to wild-type cells.

The results of the *a steX* by α *ste2* cross are listed in Table 4 with the results expected if *steX* and *ste2* are linked and unlinked. The two mutations cannot be allelic or closely linked since frequent (11/34) recombinant asci were recovered. In addition, type VI asci represent the NPD class. If they are on the same chromosome a four-strand double crossover between *steX* and *ste2* would be required.

TABLE 4
a steX (α -specific) $\times$ α *ste2* (α -specific)

Spore	Ascus phenotype									Total asci
	I	II	III	IV	V	VI	VII	VIII	IX	
A	<u>a</u>	<u>a</u>	<i>ste</i>	<u>a</u>	<i>ste</i>	<i>ste</i>	<u>a</u>	<i>ste</i>	<i>ste</i>	
B	<u>a</u>	<u>a</u>	<u>a</u>	<u>a</u>	<u>a</u>	<i>ste</i>	<i>ste</i>	<i>ste</i>	<i>ste</i>	
C	<u>α</u>	<u>α</u>	<u>α</u>	<i>ste</i>	<u>α</u>	<u>α</u>	<i>ste</i>	<i>ste</i>	<i>ste</i>	
D	<u>α</u>	<i>ste</i>	<u>α</u>	<i>ste</i>	<i>ste</i>	<u>α</u>	<i>ste</i>	<u>α</u>	<i>ste</i>	
Observed	3	1	0	0	18	2	5	3	2	34
Expected if no recombination	6	0	0	0	24	0	0	0	6	36
Expected if unlinked	1	4	4	1	16	1	4	4	1	36

Since the two mutations are in different genes, the α -specific *steX* mutation was given the gene symbol *ste3*.

Cross C: a ste3 (α -specific) by α *steX* (α -specific). This cross is analogous to cross A with α -specific mutations. Since *a ste3* mates as a wild-type *a*, it was mated with each of the other class 4 α *ste* mutants, and diploids were isolated by the low-frequency mating method. The crosses and random spore results are summarized in Table 5 for each of the α *ste* parents, with expected results based on the total number of spores in the sample. Only one α spore was recovered in all; the rare occurrence of recombination demonstrates that *ste3* is closely linked to all of the other α -specific *ste* mutations. The map distance of the VL1 *ste* from *ste3* can be calculated on the assumption that the α spore arose by recombination and not by gene conversion, while upper limits can be established for the others. Only 1/4 of the recombinants will be detectable, because of the α -specificity of the mutations. Two of the four recombinant phenotypes will still mate as *a* and one will be the α *steX steY* double mutant, which would be sterile. Only $\alpha++$ will be recovered as a recombinant, so the map distance is 4 (frequency of α) $\times$ 100. Since all of these mutations are closely linked, they likely represent only one gene.

Cross D: a steX (nonspecific) by α *steY* (nonspecific). This cross is possible only if both strains are temperature-sensitive, as are four of the class 5 *a ste* mutants (VZ2, VZ4, VAC1, and VAC2). These mutants can be mated with α strains at 23° at a high frequency similar to that of wild-type matings. Sterile α spores obtained from such crosses are also temperature-sensitive; when these are mated with the *a ste(ts)* strains at 23°, diploids can be obtained by zygote isolation or by the prototroph selection.

Many of the diploids from the first set of crosses either did not sporulate or did not have good spore viability. The viable spore samples from dissected tetrads of the four sporulating diploids are shown in Table 6, part A. The frequencies of

TABLE 5
 $a\ ste3\ (\alpha\text{-specific}) \times \alpha\ steX\ (\alpha\text{-specific})$

$a\ ste$ parent		Ratio of spores ($a:a:ste$)	Total spores	Map distance*
VB4	Observed	135: 0:136	271	< 1.5
	Expected if no recombination	135.5: 0:135.5		
	Expected if unlinked	135.5:34:101.5		
VC3	Observed	168: 0:144	312	< 1.3
	Expected if no recombination	156: 0:156		
	Expected if unlinked	156:39:117		
VL1	Observed	224: 1:265	490	0.82
	Expected if no recombination	245: 0:245		
	Expected if unlinked	245:61:184		
VQ7	Observed	53: 0:73	126	< 3.2
	Expected if no recombination	63: 0:63		
	Expected if unlinked	63:16:47		

* Map distance in centimorgans = $4\ (\text{frequency of } \alpha) \times 100$.

recombinant (mating) spores indicate that the mutation in VZ2 is unlinked to that in VAC2, and the mutation in VAC1 is unlinked to the one in VZ4. Furthermore, the absence of recombination between the mutations in VAC2 and VAC1 indicates that they are linked (less than 5.3 centimorgans) and may be allelic. However, the occurrence of two recombinants between the mutations in VZ4 and VZ2 would seem to indicate that they are linked (about 10 centimorgans) but perhaps not allelic.

Since so many of the diploids did not sporulate, the crosses were repeated. The diploids obtained were observed for sporulation and then analyzed by the qualitative random spore method described in METHODS. Although exact numbers were not determined, a minimum sample of approximately 100 spore clones was tested for each diploid. The results shown in Table 6, part B confirm the previous interpretations that the *ste* genes in VZ2 and VZ4 and those in VAC1 and VAC2 are closely linked and that the mutations in VZ4 and VAC1 and those in VZ2 and VAC2 recombine frequently and are probably unlinked. However, no mating spores were recovered from these crosses involving the

TABLE 6
 $a\text{ steX (nonspecific)} \times \alpha\text{ steY (nonspecific)}$

Origin of mutations			Ratio of spores ($a:\alpha:\text{ste}$)	Total spores	Map distance†
$a\text{ ste}$	$\alpha\text{ ste}$				
VZ2	VZ4	Observed	0:2:39	41	9.8
		Expected if no recombination	0:0:41		
		Expected if unlinked	5:5:30		
VZ2	VAC2	Observed	5:5:23	33	unlinked
		Expected if no recombination	0:0:33		
		Expected if unlinked	4:4:25		
VAC1	VZ4	Observed	7:6:27	40	unlinked
		Expected if no recombination	0:0:40		
		Expected if unlinked	5:5:30		
VAC2	VAC1	Observed	0:0:37	37	< 5.3
		Expected if no recombination	0:0:37		
		Expected if unlinked	5:5:27		

B. Qualitative random spore analysis

$a\text{ ste}$ derived from	$\alpha\text{ ste}$ parent			
	VZ2	VZ4	VAC1	VAC2
VZ2	+/-**	+/-	+/+	+/+
VZ4	+/-	+/-	-/0	-/0
VAC1	+/+	+/+	+/-	+/-
VAC2	+/+	+/+	-/0	-/0

* Spore samples were from dissected, but incomplete, tetrads, and do not represent a random sample.

† Map distance in centimorgans = 2 (frequency of a or α spores $\times$ 100).

** +/+ = The diploid sporulated; mating spores were recovered.

+/- = The diploid sporulated; no mating spores were recovered.

-/0 = The diploid did not sporulate.

mutations from VZ4 and VZ2. These mutations are certainly linked, and they are probably allelic.

The two unlinked loci represented by these mutants could be allelic with *ste2* and *ste3*, even though the phenotypes conferred by the mutations are different. Therefore, the *ste* mutation in VZ2 and VZ4 and that in VAC1 and VAC2 cannot be assigned gene numbers until they are shown to be nonallelic with *ste2* (cross E) and *ste3* (Cross F).

Cross E: a steX (nonspecific) by α ste2 (a-specific). The *a steX* strains used are VZ4 and VAC2. Since VZ4 and VAC2 are temperature-sensitive and *ste2* is *a*-specific, diploids from the *a steX* by *α ste2* crosses can be obtained by zygote isolation at 23°. The independence of *ste2* and *steX* is established by the presence of *a* spores in a small sample; therefore, few asci were dissected and only three complete tetrads were obtained from each diploid.

For the cross of VZ4 by *α ste2* one of the three asci was a recombinant ascus, while the other two were nonrecombinant asci. The existence of one recombinant in three asci establishes that *ste* from VZ4 recombines with *ste2*. In the cross of VAC2 by *α ste2*, again one recombinant ascus and two nonrecombinant asci occurred. Therefore, both nonspecific *ste* mutations are nonallelic with *ste2*.

Cross F: a ste3 (α -specific) by α steX (nonspecific). As in the previous cross, these diploids can be obtained at 23° by direct zygote isolation, since *ste3* is *α* specific and *steX* is temperature-sensitive. The *α steX* strains were derived from VZ4 and VAC2, described in crosses D and E. The occurrence of *α* spores or recombinant asci in a small sample indicates that *ste3* is not linked to *steX*.

Only half of the spores from the VZ4-derived diploid were viable with a ratio of spore types of 2 *a*:4 *α* :8 *ste*. The two *a* spores in 14 total spores provide evidence that the *ste* mutation in VZ4 recombines frequently with *ste3* and, therefore, is in a different gene. Of the five complete tetrads obtained from the VAC2-derived diploid, two were recombinant asci and three were nonrecombinant asci. The existence of recombinants in this small sample demonstrates that *ste3* is not linked to the VAC2 mutation. Therefore, VZ4 and VAC2 possess *ste* mutations that are not linked to *ste3*, *ste2* (cross E), or each other (cross D). The mutations in VZ4 and VAC2 have been designated *ste4* and *ste5*, respectively. The mutation in VZ2 is linked to *ste4* in VZ4, while the defect in VAC1 is closely linked to *ste5* in VAC2.

Summary of recombination studies: Recombination studies with some of the sterile mutations have shown that a minimum of five distinct genetic loci are represented by these mutants (Table 7). One of these is the mating-type locus or one or more genes closely linked to MT (mutant classes 1 and 2); the other loci are not centromere- or MT-linked. Centromere linkage would have been detected by second division segregation frequencies of less than 0.67. The centromere markers *leu1* and/or *trp1* were segregating in each cross. The sterile *a* mutants with *a*-specific mutations (class 3) represent a second locus, *ste2*, while the class 4 *α* mutants all contain *α* -specific mutations at another discrete site, *ste3*. These loci could each contain one or several genes with functions specific for only one mating type. Many other mutants possess defects nonspecific for mating type;

TABLE 7

Summary of genetic loci represented by the analyzed sterile mutants

Class	Mating type of parent strain	Sex factor production	Specificity of <i>ste</i>	Linkage to <i>MT</i>	<i>ste</i> locus number	Number of mutants
1	α	α -factor ⁻	unknown	close	<i>ste1</i>	10
2	α	α -factor ⁻	unknown	close	<i>ste1-5</i>	1*
3	a	a -factor ⁺	a	none	<i>ste2</i>	2
4	α	α -factor ⁺	α	none	<i>ste3</i>	5
5	a	a -factor ⁻	a or α	none	<i>ste4</i>	2
5	a	a -factor ⁻	a or α	none	<i>ste5</i>	2

* This mutant, VN33, responds to *a*-factor.

at least two loci, *ste4* and *ste5*, confer such a phenotype. These five loci included only 23 of the 93 *a* and α mutants that could form diploids at low frequency. As complementation and recombination tests are developed for the others, many more genes may be found.

These recombination studies provide additional information about the phenotypic effects of the mutations. In no case was a mutation found to have a recessive effect on sporulation ability. With the *a*-specific and α -specific mutants (*ste2* and *ste3*) this possibility was tested in diploids that were homallelic for the sterile mutations.

Analysis of nonsporulating sterile mutants: The mutants of classes 9 and 10 could not be subjected to tetrad analysis because the diploids bearing these mutations would not sporulate. In the previous paper (MACKAY and MANNEY 1974) we reported that these diploids would mate at normal haploid frequencies and have mating types opposite to those of the parent strains of the mutants. Indirect evidence that these mutants are produced by mutations of the *MT* alleles themselves was obtained by incorporating them into tetraploids. For example, the mutant VAF1 was derived from a haploid of mating-type *a*. It was mated at low frequency to a normal mating-type α strain to produce a diploid that will not sporulate, but will mate as an α . This diploid was mated with a diploid that is homozygous for *a*, to produce a tetraploid that has two normal *a* alleles, one normal α and the *MT* allele from the original mutant. Furthermore, this tetraploid is able to sporulate, producing four viable ascospores. If the original mutant carried a mutation not at *MT* that acts through a cytoplasmic product to produce the nonsporulating phenotype in the diploid, it would also be expected to produce that phenotype in the tetraploid. On the other hand, a mutation not at *MT* with a recessive action could not produce the phenotype in either the diploid or the tetraploid. We conclude that the mutant carries a defective α allele (or a linked, *cis*-specific mutation). We have tested 5 mutants from class 9 and 2 from class 10, and they all yield sporulating tetraploids. A detailed genetic analysis of these tetraploids, supporting these conclusions, will be presented in a subsequent paper.

DISCUSSION

This limited genetic analysis of nonmating mutants is sufficient to reveal some general features of the genes which, in addition to the mating type locus, are involved in the control of conjugation in this organism. In the preceding paper (MacKAY and MANNEY 1973) we have discussed the selective biases inherent in the method used to isolate these mutants. In addition, their genetic analysis is limited by the nonmating phenotype itself, which precludes constructing hybrids by conventional methods. Although most of the mutants could be crossed, at low frequency, with strains having unimpaired mating ability, it was necessary to employ indirect methods to incorporate two sterile mutations into the same diploid. With these limitations, the following generalizations are indicated by the results:

1. Each distinct phenotypic class corresponds to one or more genes (Tables 1 and 7), but we have found no case, except at the mating-type locus itself, where mutations belonging to different phenotypic classes are allelic.
2. Among the mutations that are not linked to *MT* there is a symmetry between the phenotypic classes and corresponding genes isolated in the two mating types; for every class of mutants isolated in *a* there is a parallel class that originated in α .
3. The mutations that apparently affect the mating-type alleles directly display a diversity of phenotypes, and there is an asymmetry between those affecting α and those affecting *a*. We have found three types of mutations affecting the α allele (classes 1, 2, and 10, Table 1), but only one type that affects the *a* allele (class 9, Table 1). The absence of *a* types analogous to classes 1 and 2 appears to be particularly significant.

Before a detailed molecular model of the mating-type control can be formulated it will be necessary to identify which genes are primarily involved in regulatory functions, and which ones are regulated structural genes whose products are directly involved in the metabolism of the events of conjugation and sporulation. Our results do not provide any direct evidence bearing on this. They do, however, offer some credibility to the hypothesis that the mating-type alleles are regulatory in their function and that some, if not all, of the genes that are essential for conjugation, but not linked to *MT* are structural genes for processes regulated by *MT*. It is reasonable to assume that *MT* is regulatory because of the evidence for the existence of other genes that are essential for conjugation.

The mating-type-specific mutations, *ste2* (*a* specific) and *ste3* (α specific) are most easily interpreted. These mutations affect the ability to respond to the sex factor produced by the opposite mating type, but do not affect the ability to produce sex factor, or the ability to sporulate in *a*/ α diploids. These two genes apparently control the specificity-determining step in the response to the sex factor of the opposite mating type. We can speculate that they produce gene products that mediate the response to sex factor by specifically binding with it, either at the cell surface or intracellularly. If this speculation is true, then we can also conclude that the expression of these genes is regulated by the mating-type alleles to account for the fact that a cell does not respond to its own sex factor.

We cannot rule out the possibility that these loci are complex, specifying more than a single gene product each. The mutations of classes 7 and 8 are nonspecific mutations which otherwise have the same phenotypes as *ste2* and *ste3*, respectively. However, we have not been able to test these nonspecific mutations for allelism. They may be mutations affecting aspects of the sex-factor response common to both mating types.

All other mutations that we have reported here, except one, whether linked to *MT* or not, eliminate both the ability to produce sex factor and to respond to it. Our limited genetic analysis indicates that there may be several genes in addition to *MT* which confer this mutant phenotype, and that mutations having the same phenotypic consequences may arise in either mating type. These mutations are difficult to interpret. They suggest that there exist functional or regulatory inter-relationships among these processes in addition to that defined by the mating-type alleles.

The single mutation that retains the ability to respond to a sex factor is *ste1-5*. This mutation is closely linked to *MT* and has lost the ability to produce α factor. However, as we have discussed in the previous paper (MACKAY and MANNEY 1973) the method we used to isolate these mutants selects against mutants that retain the ability to respond. We have recently isolated mutants by another method that is free of this selective bias and have recovered mutations, not linked to *MT*, that impair the ability to produce α factor, but not the ability to respond to α factor. We anticipate that these mutations define structural genes related to the biosynthesis of α factor. A detailed analysis of these mutants will be presented in a subsequent paper.

We cannot rule out the possibility that the mating-type alleles are the structural genes for their respective sex factors. However this assumption would seem to be the most difficult to reconcile with our results. Only a highly complicated and speculative model could account for the types of mutations that show close linkage to *MT* (classes 1, 2, 9, and 10). The mutations that fail to recombine with α show a hierarchy of phenotypes. Class 2 mutants have lost only the ability to produce α factor (and to mate, of course). Class 1 mutants have also lost the ability to respond to α factor, and class 10 mutants, in addition to having these defects, are defective in some function necessary for sporulation. In contrast, only one type of mutant has been found that does not recombine with the α allele—class 9 mutants, which are analogous to the class 10 mutants in α . The α mutants alone could possibly be explained on the hypothesis of a cluster, or even an operon, of related structural genes. However, the absence of mating-type α mutants analogous to classes 1 and 2, and the occurrence of the unlinked genes associated with the same functions, are difficult to reconcile with this type of hypothesis.

While our results do not yet provide enough detail for a molecular model of the mating-type control system, they do indicate a framework for understanding its general features. For purposes of discussion, genes under the control of *MT* but not linked to it will be assumed to be structural genes for conjugation and sporulation-associated functions. These could be of four types: (1) genes expressed only in α ; (2) genes expressed only in α ; (3) genes expressed in both α

FIGURE 1.—Model for mating type locus control of structural genes. Boxes denote batteries of genes; circles designate the mating-type of the cell. Induction (or derepression) is represented by solid lines with arrows; repression (or lack of induction) is indicated by broken lines with bars.

and α (and possibly also in a/α diploids); (4) genes expressed only in cells heterozygous for mating-type, a/α diploids. Throughout this discussion, the term “induction” will be used to mean expression of a gene, although derepression may be the case. “Repression” will mean lack of expression, although formally such a repressed gene may be uninduced or the product of the gene is inhibited.

A scheme for the interactions of these structural genes and the mating-type alleles is diagrammed in Figure 1. In a cells, a diffusible product (possibly a protein) of the a allele induces a -specific and nonspecific genes and may repress α -specific and a/α -specific genes. In α cells, the α product similarly induces α -specific and nonspecific sites and may repress a -specific and a/α -specific genes. In the a/α diploid, a/α -specific, and any of the nonspecific genes functional also in a/α diploids, are induced and the remainder of the nonspecific and a - and α -specific genes are repressed. It should be noted that certain nonspecific genes may be functional in all three types of cells and could, therefore, be constitutive. Mutations in these would block mating and also, when homozygous, some functions in a/α diploids. This scheme is consistent with all of the data previously described. For example, a cells produce a factor and respond to α factor, but do not synthesize α factor, respond to a factor, or sporulate. Whether these events occur by an induction mechanism or a repression system cannot be determined at present.

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