Genetic Variation of the Repeated *MAL* Loci in Natural Populations of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*

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ABSTRACT

In *Saccharomyces cerevisiae*, the gene functions required to ferment the disaccharide maltose are encoded by the *MAL* loci. Any one of five highly sequence homologous *MAL* loci identified in various *S. cerevisiae* strains (called *MAL1*, *2*, *3*, *4* and *6*) is sufficient to ferment maltose. Each is a complex of three genes encoding maltose permease, maltase and a transcription activator. This family of loci maps to telomere-linked positions on different chromosomes and most natural strains contain more than one *MAL* locus. A number of naturally occurring, mutant alleles of *MAL1* and *MAL3* have been characterized which lack one or more of the gene functions encoded by the fully functional *MAL* loci. Loss of these gene functions appears to have resulted from mutation and/or rearrangement within the locus. Studies to date concentrated on the standard maltose fermenting strains of *S. cerevisiae* available from the Berkeley Yeast Stock Center collection. In this report we extend our genetic analysis of the *MAL* loci to a number of maltose fermenting and nonfermenting natural strains of *S. cerevisiae* and *Saccharomyces paradoxus*. No new *MAL* loci were discovered but several new mutant alleles of *MAL1* were identified. The evolution of this gene family is discussed.

MALTOSE fermentation in *Saccharomyces cerevisiae* species is initiated by the transport of the disaccharide across the cell membrane by maltose permease and its cleavage into two molecules of glucose by maltase. Synthesis of both enzymes is induced by maltose and repressed by glucose and regulation occurs predominantly at the level of transcription (reviewed by NEEDLEMAN 1991).

The presence of at least one of a polygenic series of five *MAL* loci called *MAL1* (chromosome *VII*), *MAL2* (chromosome *III*), *MAL3* (chromosome *II*), *MAL4* (chromosome *XI*) and *MAL6* (chromosome *VIII*) is required for a strain to ferment maltose (reviewed in BARNETT 1981). All five loci are highly homologous on both the sequence and gene function level (CHARRON *et al.* 1989). As has been suggested for the *SUC* genes and the Y′ and X telomere-associated sequences, the repeated copies of this family of loci may have resulted from translocation events involving the telomeres of different chromosomes (CARLSON *et al.* 1985; CHARRON *et al.* 1989; LOUIS and HABER 1990; MICHELS *et al.* 1992). The underlying mechanism of this translocation remains unclear with regard to the *MAL* loci, but homologous recombination appears to be involved in the duplication of Y′ and *SUC* sequences (LOUIS and HABER 1990; CARLSON *et al.* 1985).

Each *MAL* locus is a complex of three genes. Gene 1 encodes maltose permease (CHENG and MICHELS 1989, 1991); gene 2 encodes maltase (DUBIN *et al.* 1985; HONG and MARMUR 1986) and gene 3 encodes the MAL-activator, the transcription activator which mediates maltose induction (CHANG *et al.* 1988; KIM and MICHELS 1988; SOLLITI and MARMUR 1988). All three gene functions are required for a strain to ferment maltose (NEEDLEMAN *et al.* 1984; CHARRON *et al.* 1986). The nomenclature developed to distinguish each of these gene functions at the different *MAL* loci utilizes a two digit numbering system in which the first digit indicates the locus of the gene and the second digit indicates the gene function. For example, *MAL62* encodes maltase at the *MAL6* locus. The organization of this three gene cluster at *MAL6* is shown in Figure 1.

Previously reported genetic analyses of maltose fermenting and nonfermenting strains from the wild used maltose nonfermenting natural variant strains as complementation testers and identified only two complementing gene functions at each *MAL* locus because of a lack of appropriate tester strains for all three gene functions (OSHIMA 1967; NAUMOV 1969, 1971, 1972, 1976). Even with this limitation, these studies were able to identify several naturally occurring, partially functional mutant alleles of *MAL1* and *MAL3* which lacked one or both of the two complementing gene functions. We undertook this study to broaden the scope of our genetic analysis of the *MAL* genes to natural populations and to use genetically defined complementation tester strains and the cloned *MAL* genes to ask: (1) if *MAL*-homologous sequences could be identified at chromosomal loci other than the five already identified loci, and (2) to characterize other variant alleles that

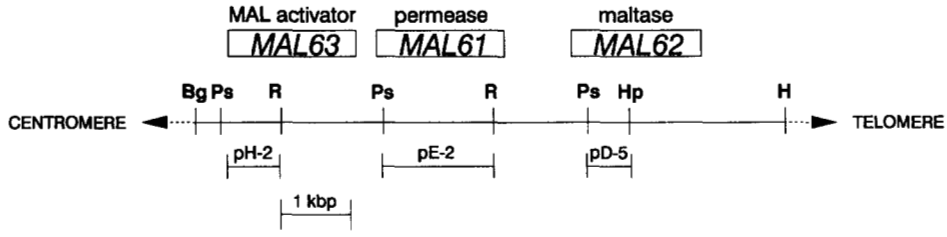

FIGURE 1.—Organization of the *MAL6* locus of Saccharomyces. A partial restriction endonuclease map of the *MAL6* locus showing the location of the *MAL61*, *MAL62* and *MAL63* genes is presented with the product of each gene indicated above the map. The yeast sequences contained in the three gene probes used in this study are diagramed below the map and all are carried by the vector plasmid pBR325. The orientation of the locus with regard to the centromere and telomere is shown. Restriction endonucleases are abbreviated as follows: Bg, *Bgl*II; H, *Hin*dIII; Hp, *Hpa*I; Ps, *Pst*I; R, *Eco*RI.

might be present in natural populations. For this we surveyed strains of *S. cerevisiae* and *Saccharomyces paradoxus* obtained from a number of sites in Europe, Asia and Africa using pulsed field gel analysis to determine the hybridizing chromosome containing *MAL6*-homologous sequences, if any, and using complementation analysis to determine which of the three gene functions were active. No new *MAL* loci were identified among the strains surveyed but the results imply the existence of previously unidentified *MAL1* and *MAL3* variant alleles in *S. cerevisiae*. Interestingly, *MAL*-homologous sequences were present but not repeated in the *S. paradoxus* species. Our work highlights the variability of this telomere-associated gene cluster.

MATERIALS AND METHODS

Strains: Tables 1 and 2, respectively, list the strains of *S. cerevisiae* and *S. paradoxus* used in this study. Indicated also are the environments from which each of the strains was obtained, the country or region of origin and any previous reference in the literature describing the strain. Strain YNN295 is the chromosomal standard obtained through Bio-Rad; fermentation data are not available for this strain (MORTIMER and CONTOPOULOU 1991). Strains X2180-1A and S288C are available through the Berkeley Stock Center collection. S288C is of particular interest as the founder strain of many American genetic lines and strain X2180-1A was obtained by sporulating a spontaneous diploid isolate from an S288C culture (MORTIMER and JOHNSTON 1986). Strain 600-1B is described in detail by CHARRON *et al.* (1986) and contains a single *MAL* locus, *MAL1*, based on Southern and genetic analysis. This strain was used to construct the genetic tester strains for the complementation analysis described below. The remaining strains are monosporic (from a single ascospore) highly fertile homothallic diploid strains derived from natural strains of *S. cerevisiae* and *S. paradoxus* isolated by us (NAUMOV 1976, 1986, 1987, 1988; NAUMOV *et al.* 1983, 1990b; Naumov and NIKONENKO 1988; NAUMOV and NAUMOVA 1991).

Complementation analysis: The compositions of the complete medium for yeast growth and the acetate sporulation medium are published previously (NAUMOV *et al.* 1986). Minimal maltose medium contains 0.67% yeast nitrogen base without amino acids and 1% maltose (filter sterilized). The ability to ferment maltose is indicated by the production of gas and acid and is determined in Durham tubes containing YEP medium plus 2% maltose.

The complementation tester strains each carry a deletion/disruption in one of the three genes comprising the *MAL1* locus and were constructed in strain 600-1B, genotype *MATa MAL1 SUC1 SUCx ura3-52 leu2-3,112* (CHARRON *et al.* 1986). The strains are referred to as: 600-1BΔ11 (*mal11Δ::URA3*), 600-1BΔ12 (*mal12Δ::LEU2*) and 600-1BΔ13 (*mal13Δ::URA3*). Crosses between the tester strains and the homothallic diploid natural strains were carried out as follows. Sporulated samples of the natural strains were treated with gluculase and the suspension of ascospores spotted onto minimal maltose plates which had been spread with cells of the tester strain. Abundant growth or a few colonies of Mal^+ hybrids were observed in 2–3 days in cases where the Mal^- parents were complementary. After 4 days of growth the mixed cultures were transferred to Durham tubes to confirm fermentation. Usually, Mal^+ hybrids fermented in 2 days but Mal^- strains failed to ferment in 9 days.

CHEF gel electrophoresis: Growth and preparation of cells for chromosomal DNA analysis has been described elsewhere (NAUMOV *et al.* 1991). A CHEF-DR™II apparatus (Bio-Rad Laboratories, Richmond, California) was used to separate the chromosomal DNAs. Electrophoresis was carried out at 200 V and 14° for 15 hr with a switching time of 60 sec and then for 8 hr with a switching time of 90 sec. A standard set of *S. cerevisiae* YNN 295 chromosomes was obtained commercially (Bio-Rad).

Southern blot analysis: After soaking the agarose gels containing the separated chromosomal DNAs in 0.25 M HCl for 5 min, the DNA was denatured and neutralized according to standard procedures (AUSUBEL *et al.* 1989) and transferred to nitrocellulose filters which were then baked at 80° for 2 hr. Transfer was carried out using a Vacuum Blotting System (Pharmacia LKB Biotechnology AB, Bromma, Sweden). Hybridization was performed in 5 × SSC containing 0.1% N-lauroylsarcosine, 0.02% SDS and 1% blocking reagent at 68° overnight after which the filters were washed twice with 2 × SSC containing 0.1% SDS at room temperature for 5 min and with 0.1 × SSC containing 0.1% SDS at 68° for 15 min. The probes were labeled with digoxigenin-11-dUTP and detection of hybridization was done using the Nonradioactive DNA Labeling Kit (Boehringer Mannheim). The filters were incubated in color solution in the dark overnight.

RESULTS

***S. cerevisiae* strains:** As a result of our molecular genetic analysis of the *MAL* loci of *S. cerevisiae* we had available DNA probes for each of the three genes required for maltose fermentation (see Figure 1) and a series of genetically defined complementation tester strains with which to investigate the genetic diversity of

TABLE 1

***S. cerevisiae* strains**

Strain	Isolation source	Geographic origin	Reference
YNN295	Genetic line		MORTIMER and CONTOPOULOU 1991
X2180-1A	Genetic line		MORTIMER and JOHNSTON 1986
S288C	Genetic line		MORTIMER and JOHNSTON 1986
N°356	Juice of *Taxus cuspidata*	Russian Far East	NAUMOV 1976
VKM Y-501	Wine	Russian Far East	NAUMOV 1976
VKM Y-502	Wine	Russian Far East	NAUMOV 1976
VKM Y-504	Wine	Russian Far East	NAUMOV 1976
VKM Y-439	Wine	Georgia	NAUMOV 1976
GM 51	Wine	Georgia	NAUMOV 1976
SBY 2576	Wine	Spain	NAUMOV 1976
GIV 51	Wine	Georgia	NAUMOV 1976
VKM Y-481	Bili Wine	West Africa	NAUMOV 1976
VKM Y-407	Ginger wine	West Africa	NAUMOV 1976
VKM Y-1232	Alpechin	Spain	NAUMOV *et al.* 1983
CBS 4054	Wine	Spain	NAUMOV *et al.* 1983
CBS 4411	Pig rectal contents	Portugal	NAUMOV *et al.* 1990b
M-427	Wine	Carpathian Mountains, Ukraine	NAUMOV *et al.* 1983
N°19	Oak exudate	Gelenjik region (Caucasus), Russia	NAUMOV 1986
N°37	Oak exudate	Novosibirsk, Russia	NAUMOV and NAUMOVA 1991
N°38	Oak exudate	Novosibirsk, Russia	NAUMOV and NAUMOVA 1991
N°39	Oak exudate	Novosibirsk, Russia	NAUMOV and NAUMOVA 1991
Ksc2	Oak exudate	Japan	NAUMOV and NIKONENKO 1988
Ksc40	Oak exudate	Japan	NAUMOV and NIKONENKO 1988
Ksc73	Oak exudate	Japan	NAUMOV and NIKONENKO 1988
600-1B	Genetic line		CHARRON *et al.* 1986
M-437	Wine	Carpathian Mountains, Ukraine	NAUMOV 1976
CCY 28-73	Wine	Slovakia	NAUMOV *et al.* 1983
VKM Y-1830	Dewberrys	Michurinsk, Russia	NAUMOV 1988
L2-43-6D	Wine	Yalta, Ukraine	NAUMOV *et al.* 1983
CBS 6006	Wine	Spain	NAUMOV *et al.* 1983
N°60	Wine	St. Petersburg, Russia	NAUMOV *et al.* 1983
M-180	Wine	Carpathian Mountains, Ukraine	NAUMOV *et al.* 1983

TABLE 2

***S. paradoxus* strains**

Strain	Mal phenotype	Isolation source	Geographic origin	Reference
CBS 432	+	Unknown		NAUMOV 1986
CBS 406	+	Oak exudate	Netherlands	NAUMOV 1986
INMIV 11/21	+	Poplar trees	Kiev, Ukraine	NAUMOV 1986
INMIV 544	–	Aspen leaves	Kiev, Ukraine	NAUMOV 1986
VKM Y-2472	+	Peat	Moscow region, Russia	NAUMOV 1986
CBS 5829	+	Mor soil	Denmark	NAUMOV 1986
N7	–	Oak exudate	St. Petersburg, Russia	NAUMOV 1987
N8	–	Oak exudate	Moscow region, Russia	NAUMOV 1987
N9	–	Oak exudate	Tashkent, Uzbekistan	NAUMOV 1987
N11	–	Oak exudate	Novgorod region, Russia	NAUMOV 1987
N12	–	Oak exudate	Lenkoran, Azerbaijan	NAUMOV 1987
N13	–	Oak exudate	Moscow region, Russia	NAUMOV 1987
N14	–	Oak exudate	Lenkoran, Azerbaijan	NAUMOV 1987
N16	+	Oak exudate	Moscow region, Russia	NAUMOV 1987
N17	–	Oak exudate	Tartastan, Russia	NAUMOV 1987
N18	–	Oak exudate	Tula region, Russia	NAUMOV 1987
N25	–	Oak exudate	Estonia	NAUMOV 1987
N34	+	Oak exudate	Voronezh, Russia	NAUMOV 1987
N36	+	Oak exudate	Lithuania	NAUMOV 1987

the *MAL* genes in natural populations. The complementation tester strains are an isogenic series of strains constructed by deletion/disruption of each of the three genes of the *MAL1* locus in a strain carrying only the fully functional *MAL1* locus, strain 600-1B (CHARRON *et al.* 1986). Each of the disruption strains is unable to ferment maltose, but each fully complements the others. Thus, we were able to identify the presence or absence of each of the three gene functions required for maltose fermentation.

The work described here includes strains from earlier studies which used natural nonfermenting variants as complementation tester strains (NAUMOV 1969, 1971, 1972, 1976). These earlier studies were limited by the

TABLE 3

Summary of genetic and Southern analysis of *S. cerevisiae* strains

Strain	Mal phenotype	Genotype[a]	Complementation analysis			Southern analysis			Genotype[b]
			mal11Δ	*mal12Δ*	*mal13Δ*	*MAL61*	*MAL62*	*MAL63*	
YNN295	ND	ND	ND	ND	ND	*II*[c]	*II,VII*	–	
X2180-1A	–	ND	ND	ND	ND	*II*	*II,VII*	–	
S288C	–	ND	ND	ND	ND				*AGT1 mal11Δ MAl12 mal13Δ MAL31 MAL32 mal33Δ*
N°356	–	*MAL1g*	–	+	–	*VII*	*VII*	*VII*	*mal11 MAL12 mal13*
VKM Y-501	–	*MAL1p*	–	–	+				
VKM Y-502	–	*MAL1p*	–	–	+	*VII*	–	*VII*	*mal11 mal12Δ MAL13*
VKM Y-504	–	*MAL1p*	–	–	+				
VKM Y-439	–	*MAL1g MAL3g*	+	+	–	*II,VII*	*II,VII*	*VII*	*MAL11 MAL12 mal13 MAL31 MAL32 mal33Δ*
GM 51	–	*MAL1g MAL3g*	+	+	–				
SBY 2576	–	*MAL1g MAL3g*	+	+	–	*II,VII*	*II,VII*	*VII*	*MAL11 MAL12 mal13 MAL31 MAL32 mal33Δ*
GIV 51	–	*MAL1p*	–	–	+	*VII*	*VII*	*VII*	*mal11 mal12 MAL13*
VKM Y-481	–	*mal0*	–	+	–	*VII*	*VII*	–	*mal11 MAL12 mal13Δ*
VKM Y-407	–	*mal0*	–	+	–	*VII*	*II,VII*	–	
VKM Y-1232	–	ND	+	+	–	*II,VII*	*II,VII*	*VII*	
CBS 4054	–	ND	ND	ND	ND	*II,VII*	*II,VII*	*VII*	
CBS 4411	–	ND	+	+	–	*II,VII*	–	*VII*	
M-427	–	ND	–	+	–				
N°19	–	ND	–	+	–				
N°37	–	ND	–	+	–				
N°38	–	ND	–	+	–				
N°39	–	ND	–	+	–				
Ksc2	–	ND	–	+	–				
Ksc40	–	ND	–	+	–				
Ksc73	–	ND	–	+	–				
600-1B	+	*MAL1*	ND	ND	ND	*VII*	*VII*	*VII*	*MAL11 MAL12 MAL13*
M-437	+	*MAL1 MAL3g*	ND	ND	ND	*II,VII*	*II,VII*	*VII*	*MAL11 MAL12 MAL13 MAL31 MAL32 mal33Δ*
CCY 28-73	+	ND	ND	ND	ND	*II,VII*	*II,VII*	*II,VII*	
VKM Y-1830	+	ND	ND	ND	ND	*II,III VII,VIII XI*	*II,III VII,VIII XI*	*II,III VII,VIII XI*	
L2-43-6D	+	ND	ND	ND	ND	*II,VII*	*II,VII*	*VII*	
CBS 6006	+	ND	ND	ND	ND	*II,VII*	*II,VII*	*VII*	
N°60	+	ND	ND	ND	ND	*II,VII*	*II,VII*	*VII*	
M-180	+	ND	ND	ND	ND	*II,VII*	*II,VII*	*VII*	

[a] Previous nomenclature.
[b] Current nomenclature.
[c] Chromosome number.

The results of the previously published genetic analysis carried out by NAUMOV (1976) are presented here using the former nomenclature. Complementation analysis of several strains was carried out using an isogenic series of *mal1* tester strains containing single deletions in each of the three *MAL* genes: *mal11Δ*, *mal12Δ*, *mal13Δ* (see MATERIALS AND METHODS). Southern analysis of size separated chromosomes was carried out using *MAL6*-derived probes from each of the MAL genes, as indicated in the Table.

fact that the natural nonfermenting variant strains carried mutations in only two of the three *MAL* gene functions. Naumov and coworkers referred to these two gene functions as *MALp* and *MALg*. Strains expressing neither function were referred to as *mal0*. Little information was available regarding the function of the *MALp* and *MALg* alleles, but analysis of a nonfermenting mutant isolated in the laboratory by TEN BERGE *et al.* (1973) suggested that the *MALp* complementation group encoded the regulatory gene function, gene 3 in the current nomenclature (NAUMOV 1976). *MALg* alleles were found linked to the *MAL1* and the *MAL3* loci, referred to as *MAL1g* and *MAL3g* respectively, and a *MALp* allele was found linked only to *MAL1*, referred to as *MAL1p*. The results of these studies using the natural complementation tester strains are presented in Table 3. In several instances, to be described below, the analysis described here has allowed us to distinguish between different variant *MAL* alleles which appeared functionally identical in the earlier studies thereby underscoring the genetic variability of the *MAL* loci.

All of the nonfermenting natural strains listed in Table 3 were analyzed using the deletion/disruption complementation tester strains. The results are consistent with the previously reported results with one exception, N°356, which we suggest might have undergone genetic change as a result of the many years of laboratory culture. Clearly, *MAL1p* expresses the gene 3 function, the MAL-activator. With the exception of the results with strain N°356, the *MALg* alleles express the

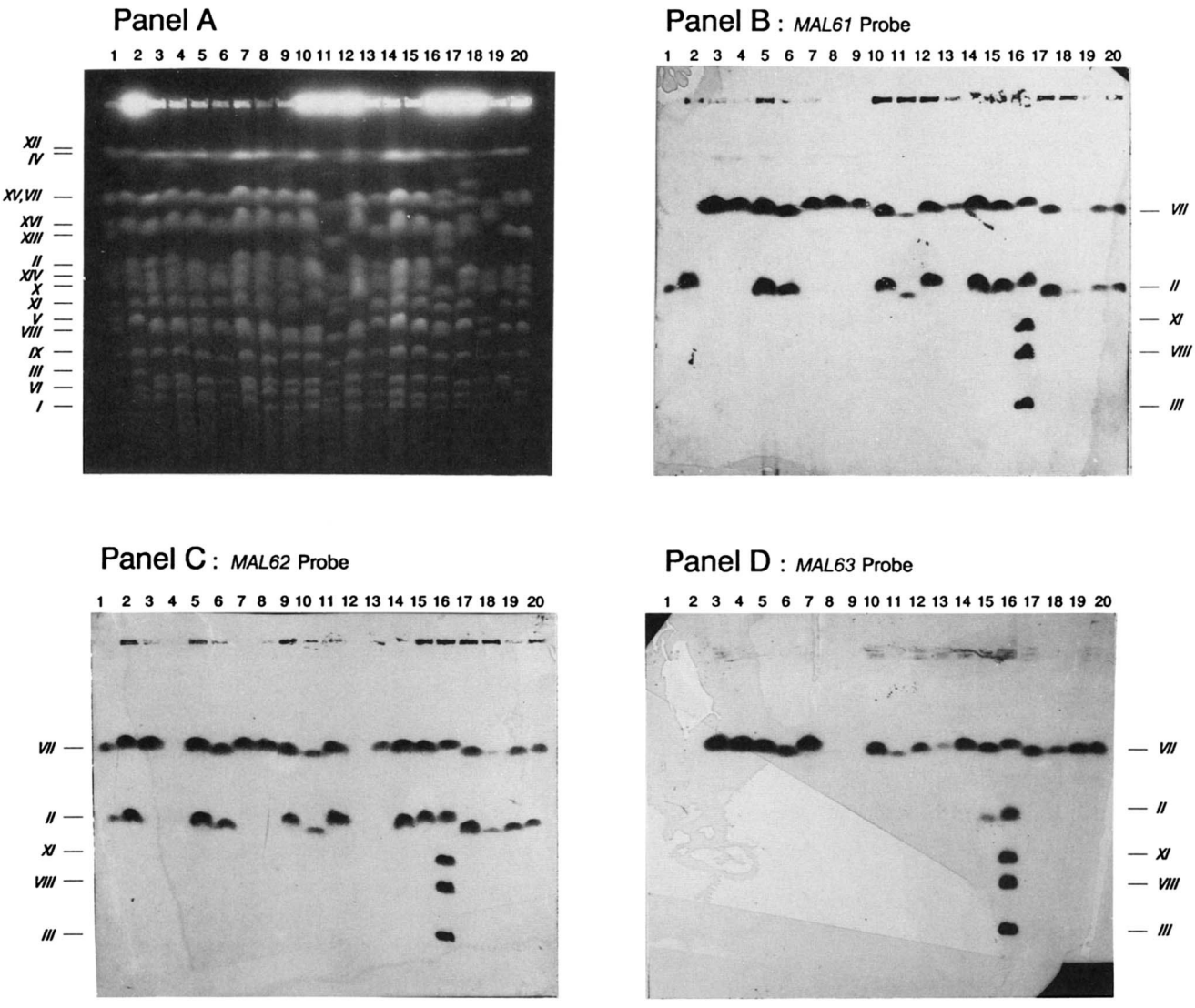

FIGURE 2.—Southern hybridization analysis of chromosomal DNAs from different *S. cerevisiae* strains. Panel A shows the electrophoretic karyotype of the strains. Panels B, C and D show the results of Southern analysis of the chromosomal DNAs probed with the indicated gene probe: B, the *MAL61* gene probe plasmid pE-2; C, the *MAL62* gene probe plasmid pD-5; D, the *MAL63* gene probe plasmid pH-2 (see Figure 1). The strains: YNN295 (lane 1), X2180-1A (lane 2), N°356 (lane 3), VKM Y-502 (lane 4), VKM Y-439 (lane 5), SBY 2576 (lane 6), GIV 51 (lane 7), VKM Y-481 (lane 8), VKM Y-407 (lane 9), VKM Y-1232 (lane 10), CBS 4054 (lane 11), CBS 4411 (lane 12), 600-1B (lane 13), M-437 (lane 14), CCY 28-73 (lane 15), VKM Y-1830 (lane 16), L2-43-6D (lane 17), CBS 6006 (lane 18), N°60 (lane 19), M-180 (lane 20).

gene 1 function, maltose permease, and the gene 2 function, maltase, as had been suggested previously (NEEDLEMAN *et al.* 1984). Strains previously designated as *mal0* can in fact be seen to encode only functional maltase, the gene 2 function, a fact gone unrecognized in studies using natural strains because of the lack of an appropriate tester strain.

Chromosomal DNA from 20 of the strains listed in Table 3 were size separated by pulsed field gel electrophoresis and the chromosomes containing *MAL*-homologous sequences identified by Southern analysis. Figure 2A shows the karyotype of the strains and Figure 2B, C and D show the hybridization patterns when probed with sequences derived from the coding region of the *MAL61*, *MAL62* and *MAL63* genes, respectively. Slight variations in karyotype can be seen, but, overall, the natural strains are very similar to the standard strain YNN295 and to strain S288C. This similarity in genomic structure is indicative of a fundamental similarity in genetic organization. Comparative analysis of karyotype using eight cloned *S. cerevisiae* genes and several *S. cerevisiae* strains, including a number of the strains used here, found that each gene mapped to a chromosome of approximately the same size in both the standard strains and the natural strains (NAUMOV *et al.* 1992). Thus, we feel we are able to identify particular chromosomes in the natural strains based on their size by comparison to strain YNN295. Unfortunately, chromosomes

VII (which carries *MAL1*) and *XV* usually run as a doublet under many electrophoresis conditions, including those used here. Therefore, we are unable to state with certainty that the hybridization to this band seen in Figure 2 is to chromosome *VII* alone and not to chromosome *XV*, or to both, but we feel confident in making this assumption for the following reasons. The results of genetic analyses of many *S. cerevisiae* strains by us and others show that functional *MAL* genes map only to the *MAL1* locus of chromosome *VII* and to the other four *MAL* loci and never to chromosome *XV*. Nonfunctional *MAL*-homologous sequences have been identified at a very limited number of genomic sites and are usually linked to *MAL1* or *MAL3* (CHARRON and MICHELS 1988; MICHELS *et al.* 1992). Additionally, in strains VKM Y-1830 (lane 16 in Figure 2) and L2-43-6D (lane 17 in Figure 2), chromosomes *VII* and *XV* migrate differently from each other, allowing us to distinguish hybridization patterns in these strains. Using a probe derived from the *ADC1* gene which lies on chromosome *XV*, we found that chromosome *XV* of strain VKM Y-1830 runs slightly faster than the standard *VII/XV* doublet of strain YNN295, with chromosome *VII* apparently migrating above it (see Figure 2A; hybridization data not shown) (AMMERER 1983). In strain L2-43-6D, chromosome *XV* runs distinctly more slowly than the standard *VII/XV* doublet of YNN295 with chromosome *VII* apparently unchanged (see Figure 2A; hybridization data not shown). The results in 2B, C and D of Figure 2 show no hybridization of the *MAL*-derived probes to chromosome *XV* in these strains and does show hybridization to chromosome *VII*. Thus, to simplify the discussion of our results we will assume that *MAL*-homologous sequences are present only on chromosome *VII*, but this assumption must be kept in mind as a possible caveat to our conclusions.

Hybridization of the *MAL*-derived probes is seen to at most two chromosomal bands, with the exception of the VKM Y-1830, where five bands are seen with all three probes. Here, these five bands correspond to chromosomes *II*, *III*, *VII*, *VIII* and *XI*, which are the chromosomes to which the *MAL3*, *MAL2*, *MAL1*, *MAL6* and *MAL4* loci respectively map, suggesting that this strain contains all five of the previously identified *MAL* loci. Complete genetic mapping of each of the loci has not been done as yet. It must be noted that strains which lack functional genes at *MAL2*, *MAL3*, *MAL4* and *MAL6* are entirely lacking all *MAL*-homologous sequences at these loci. That is to say they are null alleles as opposed to mutationally altered alleles.

In all of the strains, one, two or all three of the probes were found to hybridize to chromosome *VII* strongly suggesting that an allele of *MAL1* is universally present in *S. cerevisiae* strains. The fully functional *MAL1* allele is present in strains 600-1B and M-437 and probably in strains CCY 28-73, VKM Y-1830, L2-43-6D, CBS 6006, N°60 and M-180, based on the results of Southern analysis in these fermenting strains. A *MAL1* allele expressing the two structural genes, but lacking the regulatory gene function (previously referred to as *MAL1g*), was found in strains VKM Y-439, SBY 2576 and GM 51. Southern analysis of strains VKM Y-439 and SBY 2576 indicates that regulatory gene sequences are present on chromosome *VII*, and so the genotype of the allele in these strains can be written *MAL11 MAL12 mal13*. The structure of the *MAL1* variant allele from strains VKM Y-439 and SBY 2576 is quite different from that of a *functionally* similar *MAL1* allele found in strain S288C, in all of the maltose fermenting strains from the Berkeley Stock Center that we have studied and probably in strains YNN295 and X2180-1A, based on the results of Southern analysis reported here in Figure 2 (NEEDLEMAN and MICHELS 1983; MICHELS and NEEDLEMAN 1983, 1984). Molecular genetic characterization of the *MAL1* variant from strain S288C demonstrated that *MAL11* and *MAL13* sequences are lacking and in place of the *MAL11* gene is another functionally homologous gene which we have called *AGT1*, encoding an α-glucoside transporter with a broad substrate specificity (CHARRON and MICHELS 1988; E.-K. HAN, F. COTTY and C. A. MICHELS, unpublished data). The genotype of this allele is denoted as *AGT1 mal11Δ MAL12 mal13Δ*. Interestingly, from the results shown in Figure 2, this allele is not found in any of the natural strains used for this study.

Strains VKM Y-501, VKM Y-502, VKM Y-504 and GIV 51 contain a *MAL1* variant allele expressing the regulatory gene function (previously referred to as a *MAL1p* allele) (NAUMOV 1976). Two of these strains were included in the Southern analysis shown in Figure 2, VKM Y-502 and GIV 51. The structure of this allele in these two strains is different in that strain VKM Y-502 lacks homology to the *MAL62*-derived probe (genotype denoted as *mal11 mal12Δ MAL13*) whereas the allele in strain GIV 51 exhibits *MAL62*-homology (genotype denoted as *mal11 mal12 MAL13*). The *mal11 mal12Δ MAL13* allele of VKM Y-502 is structurally the same or similar to the structure of the *MAL1p* allele described previously (CHARRON and MICHELS 1988).

The complementation pattern in strains N°356, VKM Y-481, VKM Y-407, M-427, N°19, N°37, N°38, N°39, Ksc 2, Ksc 40 and Ksc 73 indicates that only functional maltase is expressed. Such strains were previously referred to as *mal0* strains. Figure 2 shows the results of Southern analysis on two of these strains. Strain VKM Y-481 lacks homology to the *MAL63*-derived probe but the other probes hybridized to chromosome *VII*, indicating that the genotype of this allele is *mal11 MAL12 mal13Δ*. We reported the structure of another *MAL1* allele which also expresses only maltase (CHARRON and MICHELS 1988). Unlike the allele found in VKM Y-481, all three gene sequences were present, thus the genotype is *mal11 MAL12 mal13*. The *MAL62* probe hybridized to

TABLE 4

Allelic variation of the *MAL* loci

Genotype	Reference
MAL1 alleles	
MAL11 MAL12 MAL13	CHARRON *et al.* 1986 CHARRON *et al.* 1989 This report
mal11 MAL12 mal13	CHARRON and MICHELS 1988 This report
mal11 MAL12 mal13Δ	This report
mal11 mal12Δ MAL13	CHARRON and MICHELS 1988 This report
mal11Δ MAL12 mal13Δ AGT1	CHARRON and MICHELS 1988 HAN, COTTY and MICHELS, unpublished data This report
MAL11 MAL12 mal13	This report
mal11 mal12 MAL13	This report
MAL2 alleles	
MAL21 MAL22 MAL23	CHARRON *et al.* 1989
MAL3 alleles	
MAL31 MAL32 MAL33	CHARRON *et al.* 1989 MICHELS *et al.* 1992
MAL31 MAL32 mal33Δ	MICHELS *et al.* 1992 This report
MAL4 alleles	
MAL41 MAL42 MAL43	CHARRON and MICHELS 1987 CHARRON *et al.* 1989 This report
MAL6 alleles	
MAL61 MAL62 MAL63	NEEDLEMAN *et al.* 1984 CHARRON *et al.* 1989 This report

chromosomes *II* and *VII* in strain VKM Y-407, but because a complete genetic analysis of each of the two alleles in this strain has not been done it is not possible to assign a genotype.

Most strains also showed hybridization to chromosome *II* which carries the *MAL3* locus (YNN295, X2180-1A, VKM Y-439, SBY2576, VKM Y-407, VKM Y-1232, CBS 4054, CBS 4411, M-437, CCY 28-73, VKM Y-1830, L2-43-6D, CBS 6006, N°60 and M-180). With the exception of strains VKM Y-1830, the *MAL63*-probe did not show homology to chromosome *II*. This result, along with the results of genetic analysis of some of these strains, suggests that an allele of *MAL3* is commonly present which contains the two structural genes, but lacks gene 3 sequences and function. The cloning of the same or similar alleles, previously referred to as *MAL3g*, was described by us and the genotype is best denoted as *MAL31 MAL32 mal33Δ* (MICHELS *et al.* 1992).

Taken together, the results of the previously reported genetic analysis and the results of the complementation analysis and Southern analysis presented here allow us to present the complete *MAL* genotype of several of the strains in Table 3. The different *MAL* alleles identified in this and previous studies are summarized in Table 4. Additional alleles of *MAL1* and *MAL3* were suggested by our results, but we are unable to definitively determine the complete genotype of these alleles without a more complete genetic analysis of the strains, and therefore, we have not included their genotypes in Tables 3 and 4.

***Saccharomyces paradoxus* strains:** We analyzed both maltose fermenting and nonfermenting natural strains of *S. paradoxus*. Complementation analysis indicated that all of the nonfermenting strains retained only gene 2 function and were unable to complement the *mal11Δ* and *mal13Δ* tester strains (Table 3), demonstrating that the *S. cerevisiae* MAL-activator encoded by *MAL13* is able to activate the expression of the *S. paradoxus* maltase gene. Strains VKM Y-2472, N16, N34 and N36 were delayed fermenters requiring 5–8 days to ferment maltose. The rate of fermentation in these strains could be accelerated by complementation with the *mal12Δ* tester strain, but not with the *mal11Δ* or *mal13Δ* tester strains, suggesting that the gene 1 and gene 3 activity in these strains has reduced activity or poor affinity for maltose.

Figure 3 shows the results of Southern analysis of the *S. paradoxus* strains using the *MAL6*-derived probes. As is quite clear from Figure 3A, the karyotypes of the *S. cerevisiae* strains (lanes 1–3) and the *S. paradoxus* strains (lanes 4–20) are similar. A comparison of the electrophoretic karyotype of natural isolates of these two sibling species has been reported elsewhere and clearly supports the genomic similarities (NAUMOV *et al.* 1990a; NAUMOV *et al.* 1992). In addition to the similar karyotype, the mating-type genes and the mating response pathways of *S. cerevisiae* and *S. paradoxus* are closely related and allow the two species to form hybrid diploids. We show here that interspecific complementation between the *MAL* genes also occurs. All of these results support the conclusion that the two species are closely related. In contrast, DNA/DNA reassociation data indicates only 50% homology between *S. cerevisiae* and *S. paradoxus* (VAUGHAN MARTINI 1989).

In all, of the *S. paradoxus* strains including both fermenters and nonfermenters, a single hybridizing band was seen with all three probes, with one exception. Strain N11, a nonfermenting strain, hybridized only with the *MAL62* probe. The universal hybridizing band, again, appears to be the *S. paradoxus* equivalent of the chromosome *VII/XV* doublet.

DISCUSSION

This study makes several points concerning population genetics of the *MAL* gene family and the variability of gene families mapping to telomere-linked sites. It appears that all *S. cerevisiae* strains contain an allele of *MAL1*. While a complete genetic analysis has not been done in all of the strains in this study, this conclusion is valid for the several strains where the *MAL* allele(s) has been mapped and, based on the results of the Southern analysis showing hybridization to the chromosome *VII*,

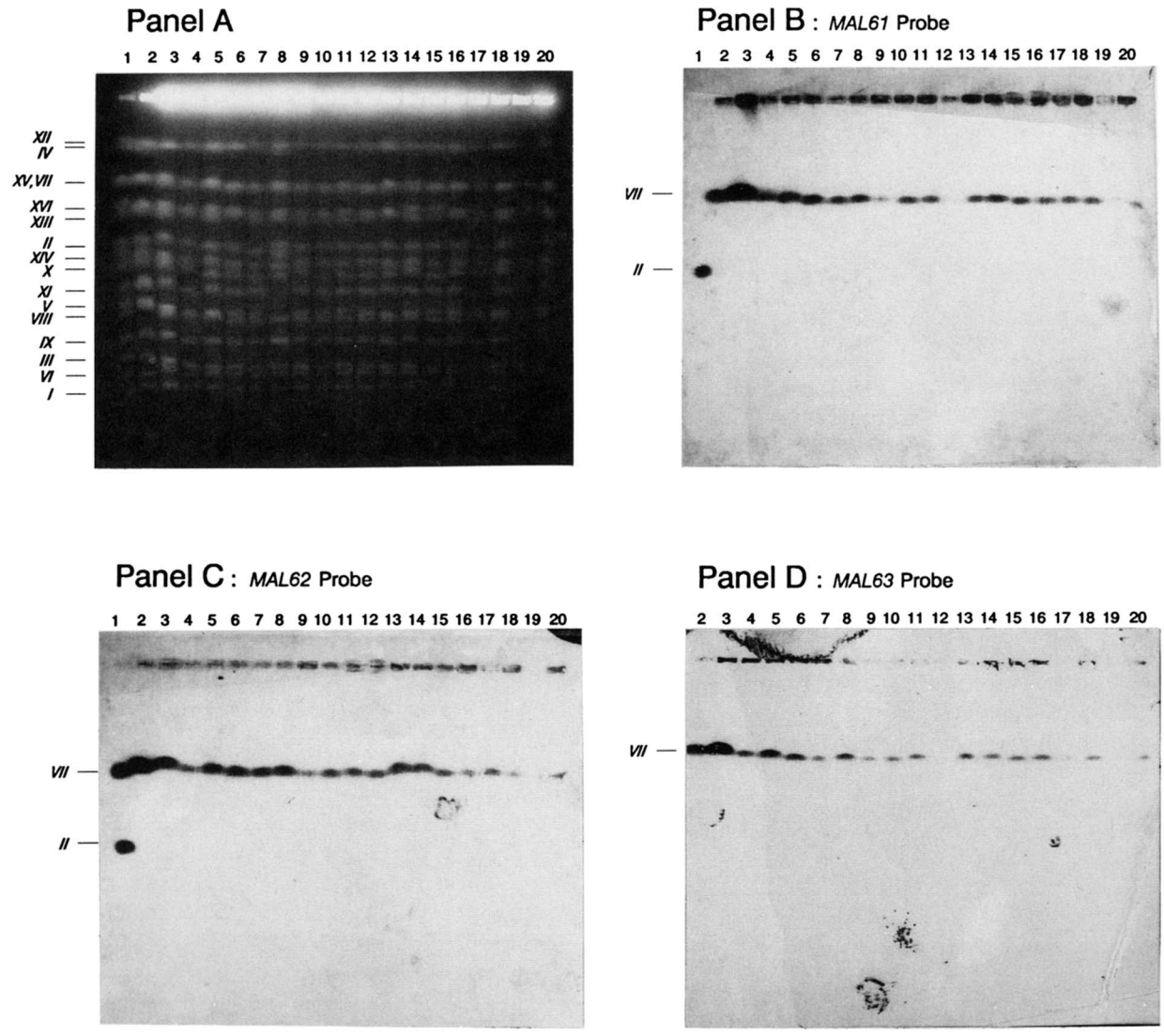

FIGURE 3.—Southern hybridization analysis of chromosomal DNAs from different *Saccharomyces paradoxus* strains. Panel A shows the electrophoretic karyotype of the strains. Panels B, C and D show the results of Southern analysis of the chromosomal DNAs probed with the indicated gene probe: B, the *MAL61* gene probe plasmid pE-2; C, the *MAL62* gene probe plasmid pD-5; D, the *MAL63* gene probe plasmid pH-2 (see Figure 1). The strains: *S. cerevisiae* strain YNN295 (lane 1), *S. cerevisiae* strain 600-1B (lane 2), CBS 432 (lane 3), CBS 406 (lane 4), INMIV 11/12 (lane 5), INMIV 544 (lane 6), VKM Y-2472 (lane 7), CBS 5829 (lane 8), N7 (lane 9), N8 (lane 10), N9 (lane 11), N11 (lane 12), N12 (lane 13), N13 (lane 14), N16 (lane 15), N17 (lane 16), N18 (lane 17), N25 (lane 18), N34 (lane 19), N36 (lane 20). Please note that in panel D the results for *S. cerevisiae* strain YNN295 (lane 1) are not presented.

we feel comfortable generalizing this conclusion. In addition, given the similarity of the *S. cerevisiae* and *S. paradoxus* karyotypes, it would appear that the sole *MAL* locus of *S. paradoxus* strains is also the *MAL1* equivalent. If so, this result suggests that *MAL1* is the progenitor locus from which the other loci were derived. It is interesting to note that the *MAL* sequences of *S. paradoxus* are not repeated in any of the strains surveyed. Possibly, selection pressures on *S. paradoxus* are different from those on *S. cerevisiae* such that multiple *MAL* genes might not be as advantageous and thus have not accumulated. Alternately, *S. paradoxus* could lack the capacity to carry out the genetic processes involved in the translocation events which created the repeated gene family or, conversely, the location of the *MAL* genes in this species precludes the translocation of the gene cluster.

It also should be noted that the *MAL1* locus, by far, shows the greatest allelic variation. This result also supports the hypothesis that *MAL1* is the progenitor of the *MAL* family of loci. Table 4 summarizes the alleles of each of the *MAL* loci which have been identified by this study and previous studies (NEEDLEMAN and MICHELS 1983; MICHELS and NEEDLEMAN 1983, 1984; CHARRON and MICHELS 1988; CHARRON *et al.* 1989; MICHELS *et al.* 1992). Other variant types are suggested from the results shown

in Figure 2 but, in the absence of a detailed genetic analysis, it is not possible to conclusively state the full genotype of these variants. Mutations in one or two of the three gene functions occur and these mutations can be simple sequence alterations not detectable by Southern analysis or restriction map analysis, deletions of the entire gene sequence and/or major rearrangements.

Approximately two-thirds of the strains surveyed here carry an allele of *MAL3*. Additionally, some allelic variation of this locus has occurred but the diversity of the alleles is not as extensive as that seen for *MAL1*. The *MAL3* locus from strain 1407-3B (Berkeley Stock Center) is a tandem array of repeated *MAL* genes (MICHELS *et al.* 1992). Two fermenting strains from this study appear to contain complete *MAL3* sequences, but the results reported here cannot distinguish whether or not the *MAL* sequences on chromosome *II* are repeated or single copy. Several of the strains in Figure 2 carry *MAL3* alleles with a deletion of gene 3 sequences, *MAL31 MAL32 mal33*Δ (previously referred to as *MAL3g*). At the level of the analysis presented here, these alleles appear identical but it is important to note that, of three *MAL31 MAL32 mal33*Δ alleles cloned from three different strains, each showed some subtle variation in the structure of the chromosomal region centromere-proximal to the locus (MICHELS *et al.* 1992).

With regard to *MAL2*, *MAL4* and *MAL6*, this study did not identify any functional variant alleles. In strains lacking *MAL2*, *MAL4* and *MAL6*, no *MAL* sequences are detectable at their locus position suggesting that the entire *MAL* locus has been deleted or, more likely, that translocation of the *MAL* genes to this chromosome has never occurred.

No new *MAL* loci were identified in the natural strains studied here. Whether this result is significant, or simply due to the small sample size, is impossible to determine. A more extensive survey of larger numbers of strains from diverse environmental and geographic origins is necessary to resolve this question.

Clearly, there is a great potential for variation in these genes. We feel this variability is associated with their proximity to the telomere. This chromosomal location is likely to be responsible for the ease of duplication by translocation seen for this and the other telomere-associated sequences, and perhaps is related to the high rate of mutation seen for *MAL1* and *MAL3*. Telomere-linked regions have been recognized in several organisms as sites for major sequence rearrangement (reviewed in LOUIS and HABER 1990 and MICHELS *et al.* 1992). The role of selection for or against high rates of maltose fermentation in the process of duplication and mutation of the *MAL* genes is not clear. On the one hand, we see multiple *MAL* genes in wine producing strains which are presumably selected based on their characteristics for the fermentation of grape juice which lacks maltose. On the other hand, we see an almost universal presence of a functional maltase gene even in strains lacking a functional MAL-activator to express it. Possibly other as yet undefined selective effects or even stocastic processes are significant factors in the evolution of this gene family.

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