

The Minisatellite MSB1, in the Fungus *Botrytis cinerea*, Probably Mutates by Slippage

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A minisatellite was identified in the intron of the ATP synthase of the filamentous fungus *Botrytis cinerea*, and it was named MSB1. This is the second fungal minisatellite described to date. Its 37-bp repeat unit is AT-rich, and it is found at only one locus in the genome. The introns of 47 isolates of *Botrytis* species were sequenced. The number of tandem repeats varied only from 5 to 11, but there were many repeat variants. The structure of MSB1 is peculiar: the variants are in the same physical order in all individuals, and this order follows the most parsimonious tree. These original characteristics, together with a total lack of recombination between alleles of the flanking regions, suggest that MSB1 probably mutates by slippage. MSB1 was found in the intron of the ATP synthase of all of the *Botrytis* species analyzed, but the repeat unit was not found in any other genus examined, including *Sclerotinia*, which is the genus closest to *Botrytis*.

Introduction

Minisatellites are tandem repeats of 6–120 bp in arrays that are 0.5–30 kb long. The first hypervariable minisatellite sequence was fortuitously isolated as a random clone from human chromosome 14 (Wyman and White 1980). Other highly polymorphic sequences were subsequently discovered near the human insulin gene (Bell 1982), α -related globin genes (Higgs et al. 1981), and the *c-Ha-ras-1* oncogene (Capon et al. 1983) and at other locations throughout the genome (Jeffreys, Wilson, and Thein 1985). However, only one minisatellite has been found in a fungus to date (Andersen and Torsten 1997). The minisatellites described by Jeffreys (e.g., Jeffreys, Wilson, and Thein 1985) are GC-rich and all have a common short core sequence, GGGCAGGAXG, repeated in tandem arrays at about 1,000 loci in the human genome. This core sequence has been widely used as a probe for DNA fingerprinting for humans (Jeffreys, Wilson, and Thein 1985), and also for rice (Dallas 1988), several gymnosperms and angiosperms (Rogstad, Patton, and Schaal 1988), birds (Wetton et al. 1987; Burke and Bruford 1987), fishes (Tagart and Ferguson 1990), and fungi (Meyer and Mitchell 1995). Several minisatellites have been found that lack the core sequence; they may be AT-rich (e.g., Yu et al. 1997; Simmler et al. 1987; Pena et al. 1995; Buresi et al. 1996; Yowe and Epping 1996) or GC-rich (Andersen and Torsten 1997). The fingerprints obtained by Vergnaud (1989) using several polymers of short oligonucleotides suggest that there may be many minisatellites without the core sequence. The numbers of tandem repeats in minisatellites can vary considerably, making them highly informative genetic markers. They are thus frequently used for genome mapping, paternity testing, population analysis, epidemiological studies, and forensic research. Processes such as strand slippage during replication (Streisinger et al. 1966; Levinson and Gutman 1987) or unequal

crossing over during recombination (Smith 1974) were first invoked for the creation of these tandem arrays and for their variability. However, only the human minisatellites have been widely studied for their variability and mode of mutation (for reviews, see Jeffreys 1997; Jeffreys et al. 1997), together with some special cases like that of the salmonids, in which the mutation processes seem to be analogous to some human processes (Withler and Miller 1997). The similarity of the core sequence to the recombination initiator χ of *Escherichia coli* and the presence of hypervariable minisatellites in the chiasmata of meiotic human chromosomes (Smith and Stahl 1985) suggested that minisatellites could function as recombination signals (Jeffreys, Wilson, and Thein 1985). However, studies of germ line length mutants at several loci showed no exchange of flanking regions (Vergnaud et al. 1991; Wolff, Nakamura, and White 1988; Wolff et al. 1989), suggesting that unequal interallelic crossing over is not the process most frequently involved in germ line mutation of human minisatellites. The MVR-PCR (minisatellite variant repeat mapping by PCR; Jeffreys et al. 1991) analysis of several human minisatellites showed polarity (Armour, Harris, and Jeffreys 1993; Neil and Jeffreys 1993; Jeffreys et al. 1994). The existence of a *cis*-acting element that activates a minisatellite allele for mutation by introducing a double-strand break which initiates an interallelic conversion process has been proposed (Jeffreys et al. 1994; Monckton et al. 1994). However, other minisatellite loci do not show polarity (Buard and Vergnaud 1994; Armour, Crozier, and Jeffreys 1996) or show intraallelic exchanges (Buard and Vergnaud 1994). Desmarais et al. (1993) have suggested that the variability of the Apo(B) AT-rich minisatellite may be due to the intrinsic properties of the repeat unit sequence. It therefore seems likely that each minisatellite has its own mode of mutation, and even a given locus does not always mutate by the same process in germ line and somatic cells (Jeffreys and Neumann 1997).

Botrytis cinerea (anamorph *Botryotinia fuckeliana*) is a haploid, filamentous, heterothallic ascomycete. It attacks a wide range of plant species in temperate regions (MacFarlane 1968) and causes gray mold on many economically important crops, such as vegetables,

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ornamentals, bulbs, and fruits, particularly grapes. In a previous study, we showed that *B. cinerea* undergoes sexual reproduction in the field and that it can be subdivided into at least two species, *B. transposa* and *B. vacuua* (Giraud et al. 1997). One of the molecular markers used for this study was the ATP synthase gene, whose PCR product shows a length polymorphism.

We analyzed the minisatellite MSB1, which has been found in the intron of the ATP synthase gene and is responsible for the variation in the length of this gene. We sequenced the minisatellite in several strains of *B. cinerea* and a few other species. This report describes the variations in length, sequence, and presence of MSB1 and compares these features with those of other minisatellites. Lastly, we suggest how the variability of MSB1 might be driven.

Materials and Methods

Isolates

A total of 44 isolates of *B. cinerea* were examined: 43 of them were collected from grapes (*Vitis vinifera*) growing in Champagne, France, and the remaining one was collected from tomatoes. One isolate of each of six other species of *Botrytis* was also analyzed (*B. calthae*, *B. fabae*, *B. convulata*, *B. pelargoni*, *B. draytoni*, and *B. alii*), three isolates of *Sclerotinia homoeocarpa*, two isolates of *Sclerotinia sclerotiorum*, and five isolates of *Sclerotinia minor*. The isolates were purified by monospore isolation and preserved in paraffin oil at 16°C. The fungi were cultured on plates containing 20 ml NY medium (20 g/l malt, 20 g/l agar, 2 g/l yeast extract) from an inoculum of 1 cm² at 25°C.

DNA Extraction

The mycelium was cultured as described by Diolez et al. (1994). Genomic DNA was isolated by the method of Möller et al. (1992).

PCR

Amplification was done in a total volume of 50 µl containing 50 ng fungal DNA, 135 ng of each primer, 100 µM dNTPs, and 0.5 U GibcoBRL polymerase in the prescribed buffer. The PCR used 30 cycles of 1 min at 95°C, 1 min at 60°C, and 2 min at 72°C. The reactions were performed in a Peltier Thermal Cycler 200 (MJ Research). The Eurogentec primers used were TGGTGAAAGAGGTGACAAC and TTTCAACGCA-GCATCAAGA for amplification of the ATP synthase gene, and AAGTTGCTGGTTCCTTGA and GTTGCA-ACCGCGTAGAT to amplify its intron.

Sequencing

PCR products were purified using the Jetsorb gel extraction kit (Bioprobe Systems) and sequenced using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase (Perkin Elmer) and the ABI PRISM™ 310 Genetic Analyser (Applied Biosystems). One strand was sequenced for each isolate, and only sequences of high enough quality were taken into account; the sequence was checked to be sure that there was no ambiguity when-

ever there was polymorphism. The GenBank accession numbers are AF020817, AF029687, AF029686, and AF020818

Southern Blots

Blotting was done as described in Giraud et al. (1997) using a purified product of PCR amplification of the intron from the isolate with 11 tandem repeats as probe.

Tree Construction

The trees of the variants were constructed using the heuristic search of PAUP 3.1.1 (Swofford 1993). Only variants without deletions or insertions of a few base pairs were taken into account for the PAUP search. The few other variants were then easily located, assuming that they were derived from the variant with the same signature but without any deletion or insertion.

Results

Isolation and Characteristics of MSB1

The ATP synthase of *B. cinerea* strain 775 was sequenced from both primers. New primers were then designed to amplify the intron, which was then sequenced. A minisatellite of 8 tandem repeats of 37 bp, one having only 28 bp, was detected (fig. 1). The minisatellite was named MSB1, for the first minisatellite of *Botrytis*. The repeat unit sequence of MSB1 did not have the core sequence and was AT-rich (62% in the consensus repeat sequence).

The introns of 47 isolates of *Botrytis* species were sequenced to determine how MSB1 varied. A total of 26 alleles were detected, each carrying 6–8 repeats except for one isolate which had 11 repeats and another which had 5 repeats. A priori, 37 different repeat unit origins could be defined. We first took the last 37 bp as the repeat unit, so that the first repeat was the shorter. The precise sequences of the repeats in MSB1 varied greatly. There were many different variants of the repeat unit sequence (minisatellite variant repeats [MVRs]), which differed from one another by point mutations (base substitutions, deletions, insertions), or the insertion of several base pairs (variants π and Δ). The 51 variants identified are listed in figure 2. A consensus tree of the full-length variants of MSB1, plus the consensus repeat unit, and a consensus tree of the shorter variants are given in figure 3. The consensus tree of the full-length variants shows two major groups of variants, b and c. The variants were named according to the tree: for each deeper branching, the letter is different (“b” or “c”; the shorter repeats were named “a”), for each second separation a first number was given, a second number indicates a third dichotomy, etc. Thus, the names of the variants indicate their similarity. For example, the names of brother variants differ only in their last numbers (e.g., “b111” and “b112”). Some variants were found in a single isolate, while others were common, being found in several species.

The alleles of MSB1 are shown in figure 4 by MVR map. The large number of repeat variants was used to detect a strong length homoplasy. Thus, 21 isolates had

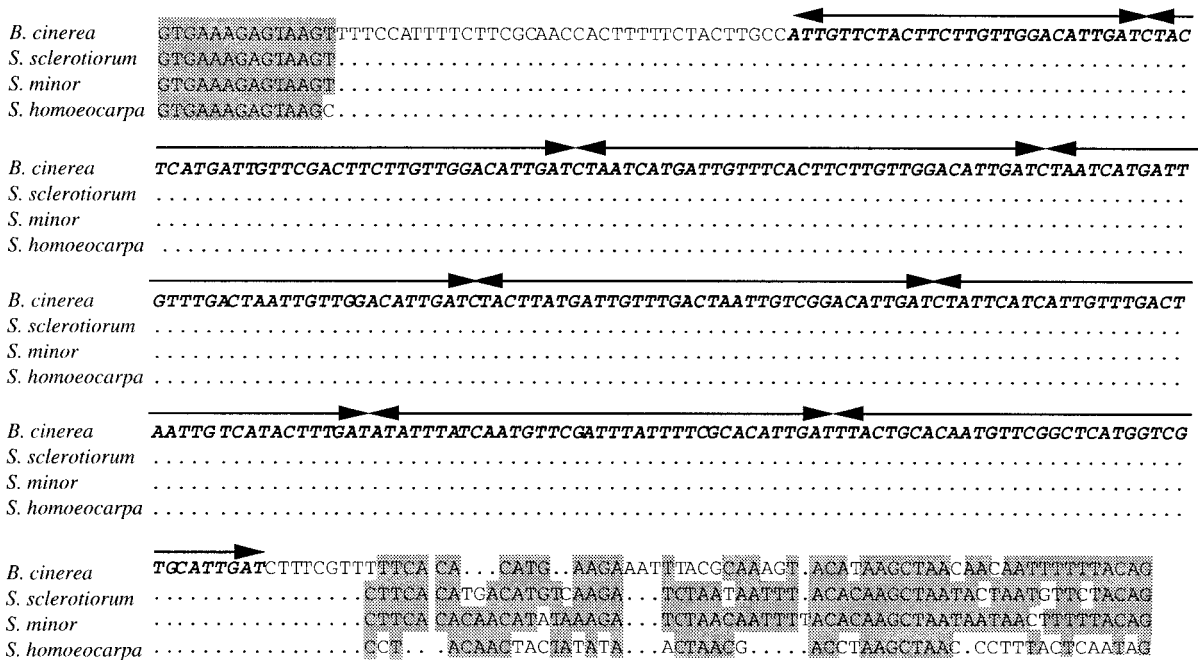


FIG. 1.—Sequences of the intron of the ATP synthase of *B. cinerea* strain 775 (GenBank accession number AF020817), the Sm5 strain of *S. minor* (GenBank accession number AF029686), the SD11 strain of *S. sclerotiorum* (GenBank accession number AF029687), and the S49 strain of *S. homoeocarpa* (GenBank accession number AF020818). Bases that are identical in at least two sequences are shaded. The minisatellite region is in bold and italic, and repeats are indicated by arrows. A gap is indicated by “.”.

8 repeats, but there were 12 distinct alleles that differed by their variants. All except 6 of the alleles were found in single isolates. These 6 were more widely represented. The variants were found in all alleles in the same physical order: there was always an a variant, followed by b variants, followed by c variants, and even within the b and c variant arrays, the order was the same in all alleles: b111 was always before b115, b1 before b2, c1 before c3, etc. Despite the fact that they were established independently, this order followed the tree of the variants: brother variants were often found at the same places, and a given variant was always near its most similar variant. a variants were found in the first position, b11x variants were found in the second and third positions, and c3x variants were always in the last positions and near the c1 and c2 variants. The same-length alleles followed the same model. The allele with 5 repeats followed the model “abbcc,” all alleles with 6 repeats followed the model “abbbcc,” the alleles with 7 repeats had the structure “abbbcc” or “abbbcc,” the alleles with 8 repeats had the structure “abbbbbcc” or “abbbbbcc,” and lastly, the allele with 11 repeats had the structure “abbbbbbcc.”

In order to be sure that the strict physical order of the variants was not an artifact due to the arbitrary definition of the repeat unit, and to check that the tree indeed reflected some reality, the same work was done with another repeat unit definition (data not shown). The last repeat was chosen to be the shortest in each allele, so the repeat unit definition was the first 37 bp of MSB1. The consensus of the most-parsimonious trees of these new variants was constructed, and names were given to the variants according to this tree as previously de-

scribed. The new MVR maps showed that variants were again in the same physical order in all alleles, and, again, the most similar variants were the most physically close. This indicates that these two features of MSB1 do not depend on the definition of the repeat unit. It means that “signatures” in the repeat are found in the same order along all alleles, and that the most similar signatures are also the most physically close.

We looked for polymorphism in the 100-bp flanking regions of MSB1 in 23 isolates. The seven base pairs that were informative are represented in figure 5. In all isolates, the seven polymorphic base pairs were associated: 5 isolates had the alleles H, I, J, K, L, and M, and 18 isolates had the alleles h, i, j, k, l, and m. This indicates that the whole region behaves as a single linkage group. The total lack of recombination between the flanking regions could be due to the species barrier between *B. transposa* and *B. vacuua*. However, we found both alleles in both species.

MSB1 in Other Genera

MSB1 was hybridized with genomic DNA from several isolates by Southern blotting, but only one locus per strain, the ATP synthase gene, was detected (data not shown). No fingerprints like those found with core minisatellites were obtained. MSB1 was then hybridized with genomic DNA from different species. The genomes of several individuals from each of four species of fungus (*Magnaporthe grisea*, *Podospora anserina*, *Fusarium oxysporum*, and *Leptosphaeria maculans*) and from *Arabidopsis thaliana* and *Drosophila simulans* were assayed. No hybridization signal was detected. MSB1 was

Variant	Sequence
Cons.	CTACTCATCATTGTPCGACTTATTGTCGGACATTGAT
a11T...C...T.....
a12T...C. A...T.....
a13T...C...T.....
a2T...C...T...T.....
b111G.....C...T.....
b112	..A...G.....TC...C...T.....
b113G.....TC...C...T.....
b114	..A...G.....C...T.....
b115	..A...G.....T...C...T.....
b116G.....T...C...T.....
b117G.....T...C...TA.....
b118G.....T...T...T.....
b12	..A...G.....T...A...T.....
b13	..A...G.....T...A.....
b14G.....T...A...T.....
b15G.....T...A.....
b16	..A...G.....TG.....
b17111	..T.....T...A...A...T.....
b17112	..T.....T...A...A...T.....
b1712	..T.....T...A...AT...T.....
b172	..T...G.....T...A...A...T.....
b173	..A...G.....T...A...A...T.....
b174	..A...G.....T...A...AT...T.....
b1751	..T...G.....A...AT...T.....
b17521T.G.C.....A...A...T.....
b17522T.G.....A...A...T.....
b1753G.....A...A...T.....
b176G.....T...A...AT...T.....
b2T.G.....T...A.....
b3T.....T...A.....
c11	A..T.T...A.....T...T.....
c121	A..T.T...A.....T...T...C.....
c122	A..T.T...A.....T...T...C.....
c13	A..T.T...A.....T...T...C...C...
c14	A..T.T...A.....T...T...C...T...
c151	T.GT.T...A...T...T...T...T.....
c1521	T.GTGT...A...T...T...T...T.....
c1522	T.GTGT...A...T...T...T.....
c2	T...TGA.....T.....T...T.....
c31	T...G..G.A.....G..C..G...TG.....
c321	T...GCA..A.....G..C..G...TG.....
c3221	T...GCA..A.....G..C..G...TG.....
c3222	T...GCA..A.....G..C..G...TG.....
c3231	T...GCA..A.....G..CG..G...TG.....
c3232	T...GCA..A.....G..CG..G...TG.....
c331	T...T.A.....G.GC..G...TG..C...
c332	T...TCAA.....G.GC..G...TG..C...
c333	T...TGA.....G.GC..G...TG..C...
Δ
∂
Π	T...

FIG. 2.—Sequences of the variants. The match with the consensus variant is indicated by “.”, a gap is indicated by “-”, and an insertion is aligned below its preceding base pairs.

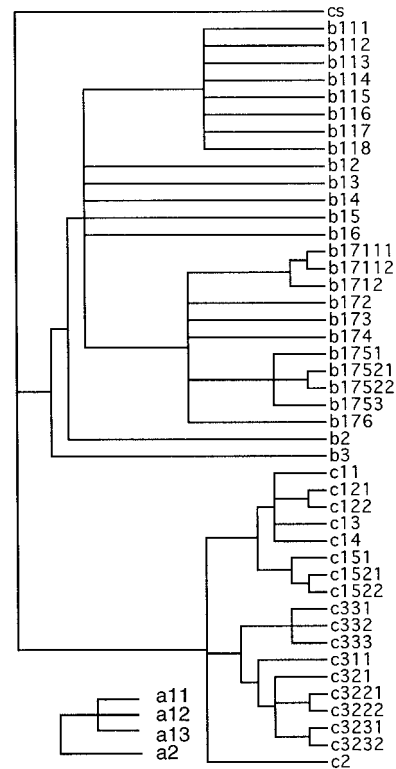


FIG. 3.—Unrooted trees of the different repeat variants: consensus tree of the shorter variants (“a” variants) and consensus tree of the 66 most-parsimonious trees of the full-length variants (“b” and “c” variants).

compared with the GenBank database, but no significant similarity with any other sequence was found.

The introns of the ATP synthases from two isolates of *S. sclerotiorum*, three isolates of *S. minor*, and three isolates of *S. homoeocarpa* were amplified and sequenced (fig. 1). The intron was short in these 8 isolates and lacked MSB1. Some base pairs at each end of the intron were similar to the sequence of *Botrytis*, but the central parts of the introns completely lacked MSB1 and also lacked 36 bp before and 8 bp after the minisatellite. The repeat unit sequence could not be found in any part of the introns of the *Sclerotinia* species.

Discussion

MSB1 Is a Novel Minisatellite

The most remarkable and unusual features of MSB1 are (1) the fact that variants are in the same order in all strains and (2) the parallel between this physical order of the variants and their most parsimonious tree. This strict order of variants has not been found in any other well-described minisatellite. The MVR-PCR technique showed variants completely mixed, and the orders of the variants were completely different in the alleles (e.g., Jeffreys et al. 1991).

Slippage as the Cause of the Variations in the Length of MSB1?

The lack of flanking-region exchange, the strict physical order of the variants, and its coincidence with

Allele number	Nb of repeats	MVR map								Nb of isolates						
		T	V	O												
1	5	a ₂	b ₁₁₈	b ₁₇₆	c ₁₂₁	c ₃₂₁						1 ^a				
2	6	a ₁₁	b ₁₁₁	b ₁₁₅	b ₁₇₃	c ₁₂₁	c ₃₂₁						1			
3		a ₁₁	b ₁₁₁	b ₁₁₅	b ₁₇₄	c ₁₂₁	c ₃₂₃₁						1			
4		a ₁₁	b ₁₁₁	b ₁₁₅	Δ	b ₁₇₃	c ₁₂₁	c ₃₂₃₁						9		
5		a ₁₁	b ₁₁₁	b ₁₁₅	Δ	b ₁₇₃	c ₁₂₁	c ₃₂₃₁₂						1		
6		a ₁₁	b ₁₁₁	b ₁₁₅	Δ	b ₁₇₃	c ₁₄	∂	c ₃₂₃₁						1	
7		7	a ₁₂	b ₁₁₁	b ₁₂	b ₁₇₅₃	c ₁₅₂₂	c ₂	c ₃₃₁						1	
8	a ₁₁		b ₁₁₁	b ₁₆	b ₃	b ₁₇₁₁₁	c ₁₁	c ₃₂₁						2		
9	a ₁₁		b ₁₁₆	b ₁₁₅	b ₁₅	b ₁₇₁₁₁	c ₁₂₁	c ₃₂₁						1		
10	a ₁₁		b ₁₁₆	b ₁₁₅	b ₁₇₅₂₂	c ₁₅₂₂	c ₂	c ₃₃₁						1		
11	a ₁₁		b ₁₁₆	b ₁₂	b ₁₇₅₂₂	c ₁₅₂₁	c ₂	c ₃₃₁						1		
12	a ₁₁		b ₁₁₇	b ₁₄	b ₁₇₅₁	b ₁₇₁₁₁	c ₁₁	c ₃₂₁						4		
13	a ₁₁		b ₁₁₇	b ₁₄	b ₁₇₅₁	c ₁₅₂₂	c ₂	c ₃₃₂						1		
14	8	a ₁₁	b ₁₁₁	b ₁₁₂	b ₁₂	b ₂	b ₁₇₁₁₁	c ₁₁	c ₃₁						1	
15		a ₁₁	b ₁₁₁	b ₁₁₂	b ₁₂	b ₂	b ₁₇₁₂	c ₁₁	c ₃₂₁						2	
16		a ₁₁	b ₁₁₁	b ₁₁₂	b ₁₂	b ₂	b ₁₇₁₁₁	c ₁₂₁	c ₃₂₁						4	
17		a ₁₁	b ₁₁₁	b ₁₁₂	b ₁₂	b ₂	b ₁₇₁₁₂	c ₁₃	c ₃₂₁						2	
18		a ₁₁	b ₁₁₁	b ₁₁₃	b ₁₂	b ₂	b ₁₇₁₁₂	c ₁₁	c ₃₂₁						1	
19		a ₁₁	b ₁₁₁	b ₁₁₂	b ₁₂	b ₂	b ₁₇₁₂	c ₁₂₁	c ₃₂₂₂						1	
20		a ₁₃	b ₁₁₁	b ₁₁₂	b ₁₂	b ₂	b ₁₇₁₁₁	c ₁₂₁	c ₃₂₁						1	
21		a ₁₁	b ₁₁₁	b ₁₁₄	b ₁₁₅	b ₁₃	b ₁₇₁₁₁	c ₁₂₁	c ₃₂₁						2	
22		a ₁₁	b ₁₁₁	b ₁₁₄	b ₁₁₅	b ₁₃	b ₁₇₁₁₁	c ₁₂₂	c ₃₂₁						1	
23		a ₁₁	b ₁₁₁	b ₁₁₅	b ₁₂	b ₂	b ₁₇₁₂	c ₁₂₁	c ₃₂₁						1	
24		a ₁₁	b ₁₁₁	b ₁₁₂	b ₁₂	b ₂	b ₁₇₂	c ₁₂₁	c ₃₂₂₁						1	
25		a ₁₁	b ₁₁₇	b ₁₁₇	b ₁₂	b ₁₇₂	c ₁₅₁	c ₂	c ₃₃₃						1	
26	11	a ₁₁	b ₁₁₆	b ₁₁₆	b ₁₁₆	b ₁₁₆	b ₁₁₆	b ₁₄	b ₁₇₅₂₁	c ₁₅₁	Π	c ₂	c ₃₃₁			1

FIG. 4.—The allele number for each allele of MSB1 detected and the number of tandem repeats, MVR map, and number of isolates of *B. transposa* (T), *B. vacuina* (V), and other species of *Botrytis* (O) that carry it. ^a *B. fabae*, ^b *B. calthae*, ^c *B. convulata*, ^d *B. cinerea* on tomatoes.

the most parsimonious tree all suggest how the instability at this locus is driven. The total lack of recombination in 23 isolates between the alleles of the flanking regions of MSB1 (fig. 5) clearly shows that unequal crossing over cannot be the major cause of mutation in MSB1, although genetic recombination is frequent in *B. cinerea* in Champagne (Giraud et al. 1997).

The variability of MSB1 could then be due to interallelic conversion like that described by Jeffreys et al. (1994) and Monckton et al. (1994), conversion between two sister chromatids, or slippage. However, only a mechanism that adds or removes a single repeat at a time, and adds this repeat near the most similar one, can produce the observed conserved order of variants, with the resembling repeats being physically close to each other. This does not seem consistent with a conversion mechanism. To conserve the same order of the variants in all alleles by interallelic conversion, we would have to assume that there is always an alignment between two identical variants, then a DNA break just after this variant, and then interallelic conversion of a single repeat. Furthermore, even these very strong assumptions would change the order of the variants if there was conversion

between certain alleles (e.g., alleles 3 and 22). All the conversion events described so far in minisatellites have involved several repeats (e.g., Jeffreys et al. 1994; Monckton et al. 1994), even for the most simple somatic events (Jeffreys and Neumann 1997). Moreover, conversion should homogenize repeats, so the great number of variants of MSB1 and the fact that all variants are different in almost all alleles do not support this mechanism. The conversion between two sister chromatids assumes another complicated mechanism: the two allele copies should be perfectly aligned, with a single repeat shift; otherwise there would be no change or the order of the variants would change. Appelgren, Cederberg, and Rannung (1997) suggested an alignment at the 5' end between two different alleles, but if a perfect alignment of the 5' flanking region is conceivable, a constant single-repeat shift seems to be very speculative.

The simplest explanation that fits all our results is that mutations leading to a change in the number of repeats are rare and occur only by one-repeat slippage on the same chromatid. The new replicated strand slips and is mispaired with the homologous part of the preceding repeat. The mutations leading to a change in the

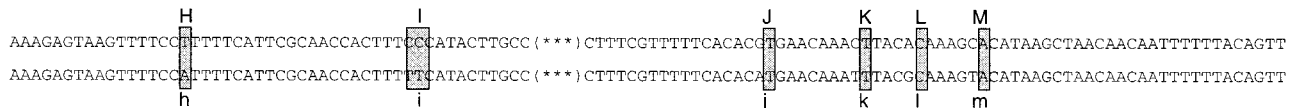


FIG. 5.—Polymorphism in the flanking regions of MSB1. The seven base pairs that are polymorphic and informative are in the shaded boxes. “***” represents the minisatellite. In 23 isolates, no recombination was observed between these seven base pairs.

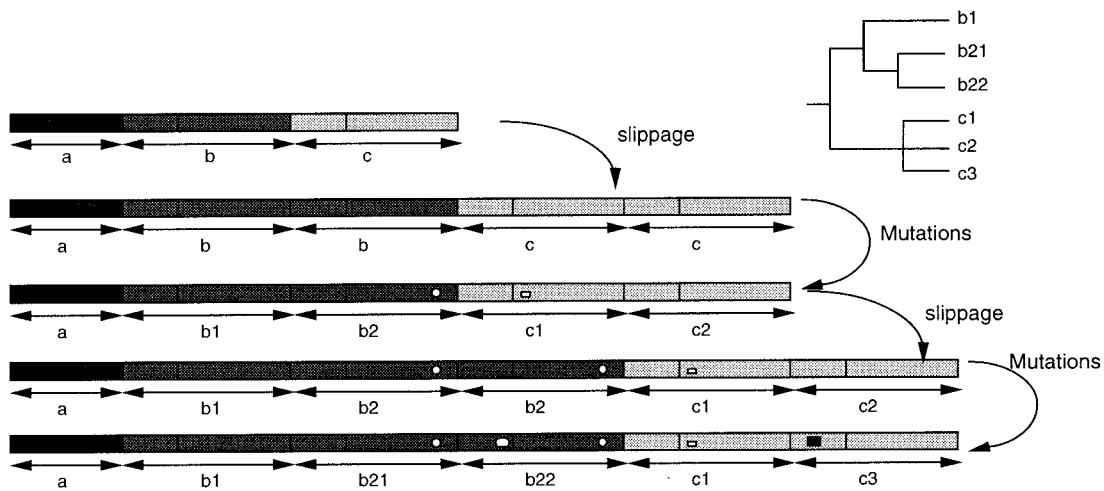


FIG. 6.—Model of the generation of variability in MSB1 by slippage.

number of repeats must be rare to explain the scarcity of alleles of different lengths and to allow time for new repeats to mutate, leading to the appearance of many new variants, each often restricted to a single isolate. The fact that most variants that resemble each other are often found at the same place and near their closest variant also points to this mechanism, as illustrated in figure 6: variants are duplicated in tandem; point mutations occur, so the son variant is always near its father variant. The tree would then really be a phylogenetic tree. The unresolved branches of the variant tree may result from new son variants arising independently from the same father variant (e.g., c1, c2, and c3 variants in fig. 6). Footprints of mutations are still visible in the MVR maps (fig. 4); e.g., the 11 repeats of the 26th allele seem to be due to four tandem duplications of the b116 variant. The fifth allele seems to have arisen from the fourth allele by a single point mutation in the last repeat. The six “abbbcc” repeats can be generated from the “abbc” of the five-repeat alleles by tandem duplications of a b variant. The patterns “abbbbcc” or “abbbccc” followed by the alleles with seven repeats could arise from the six-repeat pattern “abbbcc” by duplication of a b or a c variant. It is noticeable that b variants seem to be preferentially duplicated.

The length of MSB1 seems to be under some constraint. Our 356 isolates from Champagne included no allele shorter than 6 repeats except for the single allele with 5 repeats, and no alleles with more than 8 repeats except for the single 11-repeat allele. The upper limit could be due to a physical constraint, such as the intron

being spliced. However, the 11-repeat allele exists, and it appears to be a recent allele, because its five b116 variants do not yet have any mutations. This allele, along with the fact that some minisatellites are very long even in introns, suggests that the upper constraint could be a time constraint. MSB1 is found only in the *Botrytis* genus, and the rare mutations may not yet have produced long alleles.

The Origin of MSB1

The absence of MSB1 from the genomes of *M. grisea*, *P. anserina*, *F. oxysporum*, and *L. maculans* and from the intron of the ATP synthase of several *Sclerotinia* species, one of which is the fungus closest to *Botrytis* (Carbon and Kohn 1983), indicates that this minisatellite appeared very recently, in the branch leading to the *Botrytis* genus (fig. 7). The lack of any sequence in the intron of *Sclerotinia* that could resemble the repeat unit of MSB1, the lack of hybridization at any other locus in the *B. cinerea* genome, and the lack of any similar sequence in published databases make the origin of MSB1 very intriguing. MSB1 must have arisen after the speciation between *Sclerotinia* and *Botrytis*. The fact that the most common variants are found in different *Botrytis* species indicates that it arose well before the speciations within the *Botrytis* genus. Minisatellites present in a single genus, or even in a single species, have been found in *Salmoninae* and in *Salmonidae* (Goodier and Davidson 1998), and some minisatellites have been shown to be highly variable in only one species (Gray and Jeffreys 1991). Such loci could give indications about how minisatellites are generated.

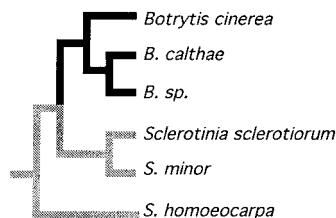


FIG. 7.—Phylogenetic tree of the species analyzed. Black indicates the presence of the minisatellite MSB1.

Conclusions

Several different mechanisms have been proposed to explain the mutant structures or natural allelic polymorphism in different minisatellites. The characteristics of MSB1 differ from those of all other minisatellites that have been thoroughly analyzed. These original features strongly suggest that MSB1 mutates by slippage, although other mechanisms, such as conversion, cannot

be totally excluded. The origin of MSB1 remains unknown, but the fact that it is present in only one genus is interesting.

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