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Inheritance of Chromosome-Length Polymorphisms in *Coprinus cinereus*

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ABSTRACT

We have investigated the inheritance of chromosome-length polymorphisms in the basidiomycete *Coprinus cinereus*. The electrophoretic karyotypes of interfertile strains of *C. cinereus* are strikingly different, and crosses between strains with different karyotypes yield progeny with chromosomes of new sizes. Repeated backcrossing of a mutant to one parent often stabilizes the mutant chromosome at a unique size; this then becomes a chromosome-length polymorphism marker for that mutant gene. A comparison of mutant strains, their wild-type progenitor, and backcrossed strains revealed that these marker chromosomes are not caused by the initial mutagenic treatment and are found only in progeny of crosses between strains with polymorphic chromosomes. Thus, they are most likely formed by meiotic recombination. For the *rad12* gene, the marker chromosome can further recombine to become the size of the homolog of the backcross parent. For the *rad3* gene, both ectopic and homologous recombination events are likely involved in the generation of the marker chromosomes. As predicted by a recombination model, a cross to a new wild-type parent can change the size of a mutant marker chromosome. Therefore, changes in chromosome length are a common and prominent feature of the genome of this sexual fungus, and a variety of karyotypes is tolerated by the organism.

THE chromosomes of most fungi are small enough to be separated using techniques of pulsed field gel electrophoresis (reviewed in MILLS and MCCLUSKEY 1990; MORTIMER *et al.* 1990; SKINNER *et al.* 1991). For many fungi, the electrophoretic analysis of chromosomes has allowed the discovery of extensive chromosome-length polymorphism (CLP; MILLS and MCCLUSKEY 1990; MORTIMER *et al.* 1990; SKINNER *et al.* 1991; PUKKILA and CASSELTON 1991; ZOLAN *et al.* 1992). This polymorphism may reflect genetic translocations, and several cases of known translocations have been reported, including the well documented *Tox1* translocation in *Cochliobolus heterostrophus* (TZENG *et al.* 1992), in which a breakpoint for a reciprocal translocation maps to the locus of the virulence gene *Tox1*. However, in many cases the CLPs are of unknown origin, and appear to have limited genetic consequences, since many different karyotypes are viable.

In addition to polymorphism of the A chromosome set, dispensable B chromosomes have been found in several fungi. In *Nectria haematococca* the B chromosome is known to contain the *Pda6* gene, which is involved in host range (MIAO *et al.* 1991; KISTLER and MIAO 1992), whereas in the rice blast fungus *Magnaporthe grisea* no phenotype has been associated with this extra genetic material (VALENT and CHUMLEY 1991).

All examined strains of the basidiomycete *Coprinus cinereus* have 13 A chromosomes, which range in size from one to 5 megabases (Mb), and no known B chromosomes. The *C. cinereus* electrophoretic karyotype is highly polymorphic among different interfertile strains (PUKKILA and CASSELTON 1991; ZOLAN *et al.* 1992). Although viable random spore progeny are readily obtained in outcrosses of *C. cinereus* strains, the viability of outcross tetrads is low (PUKKILA 1992) indicating that at least some length polymorphism reflects genetic translocations. In contrast to the observed variation among strains, different *C. cinereus* karyotypes are stable mitotically, with the exception of the chromosome containing the tandem repeat of ribosomal RNA genes (rDNA; PUKKILA and SKRZYNIA 1993). This chromosome varies in size, and its variation has been correlated with changes in the number of rDNA repeats.

In contrast to the mitotic stability of the karyotype, meiosis between strains with different karyotypes yields progeny with novel chromosome sizes (ZOLAN *et al.* 1993). In this paper we show that the examination of electrophoretic karyotypes of backcrossed mutant strains allows the correct assignment of mutant *rad* genes to chromosomes. An analysis of the generation and maintenance of marker chromosomes indicated that these novel-sized chromosomes are most likely formed by meiotic recombination, both between homologs of different sizes and by ectopic pairing.

MATERIALS AND METHODS

Strains and culture conditions: The wild-type strain Java-6 (BINNINGER *et al.* 1987) was used for mutant isolation, and mutants were isolated and backcrossed to the wild-type strain Okayama-7 (WU *et al.* 1983) as previously described (ZOLAN *et al.* 1988). Radiation screens and strain maintenance were also as previously described (ZOLAN *et al.* 1988). Strains analyzed in this study are described in full in Table 1.

Chromosome plugs and pulsed field gel electrophoresis: Plugs of intact *C. cinereus* chromosomes were prepared as

TABLE 1

Strains

Strain/Strain group	Figure(s)	Genotype/Description	Source/Reference
Java-6 (J6)	1–4, 7–9	Wild-type	BINNINGER *et al.* (1987)
Okayama-7 (O7)	1–4, 6–8	Rad$^+$	WU *et al.* (1983)
MZC3	Table 2	Wild-type	ZOLAN *et al.* (1992)
rad3$^+$ isolates	2	Rad$^+$ progeny of the cross: 3-1;4-29 × O7	This study
3-1 original	4	*rad3-1*, Rad$^-$ derivative of Java-6	ZOLAN *et al.* (1988)
3-1;4-29	2	*rad3-1*/Rad$^-$ isolate from 4th generation backcross of *rad3-1* to O7	This study
rad3-1 isolates	2	Rad$^-$ progeny of the cross: 3-1;4-29 × O7	This study
3-1;5-2; 3-1;5-9	8, 9	*rad3-1*/Rad$^-$ isolates from 5th generation backcross of *rad3-1* to O7	This study
3-2;5-4	4	*rad3-2*/Rad$^-$ isolate from 5th generation backcross of *rad3-2* to O7	This study
3-3 original	4	*rad3-3*/Rad$^-$ derivative of Java-6	This study
3-3;5-8	4	*rad3-3*/Rad$^-$ isolate from 5th generation backcross of *rad3-3* to O7	This study
3-4 original	4, 7, 8	*rad3-4*/Rad$^-$ derivative of Java-6	This study
3-4 first generation sibling isolates	7	Rad$^-$ progeny of the cross: 3-4 original × O7	This study
3-4;4-1	8	*rad3-4*/Rad$^-$ isolate from 4th generation backcross of *rad3-4* to O7	This study
3-4;5-10, 3-4;5-13, 3-4;5-15	4, 8	*rad3-4*/Rad$^-$ isolates from 5th generation backcross of *rad3-4* to O7	This study
rad9-1 sibling isolates	1	*rad9-1*/Rad$^-$ isolates from 5th generation backcross of *rad9-1* to O7	This study
rad11-1 isolates	3	*rad11-1*/Rad$^-$ isolates from 5th generation backcross of *rad11-1* to O7	This study
12-3;5-5	6	*rad12-3*/Rad$^-$ isolate from 5th generation backcross of *rad12-3* to O7	This study
rad12 6th generation sibling isolates	6	*rad12-3*/Rad$^-$ isolates from 6th generation backcross of *rad12-3* to O7	This study

Tetrads (Figures 8 and 9) are described in the figure legends.

described (ZOLAN *et al.* 1992) and analyzed using Bio-Rad clamped homogeneous electric field (CHEF; DRII or Mapper) electrophoresis equipment. Conditions for CHEF electrophoresis were as described (ZOLAN *et al.* 1992, 1993). All gels shown were run in 0.5 × TBE buffer at 14°. All of the gels shown in this paper were run using Beckman low endoosmosis agarose.

Restriction fragment length polymorphism (RFLP) analysis: RFLP analysis was performed as previously described (ZOLAN *et al.* 1992, 1993).

Hybridizations: Gels were blotted onto Zetabind (AMF Cuno) or Nytran (Schleicher & Schuell) and hybridized using either a Genius non-radioactive DNA detection kit (Boehringer Mannheim; Figure 3) or probes prepared by random-primed incorporation of [^{32}P]dATP to specific activities of 10^8 to 10^9 cpm/μg (SAMBROOK *et al.* 1989).

RESULTS

CLP markers in backcrossed mutant strains: We previously isolated radiation-sensitive (*rad*) mutants by treating oidia of strain Java-6 with ultraviolet light and screening surviving cultures for sensitivity to ionizing radiation (ZOLAN *et al.* 1988; G. VALENTINE, Y. WALLACE, J. FRIEDMAN and M. ZOLAN, manuscript in preparation). Mutants thus identified were crossed and backcrossed to strain Okayama-7, a vigorous Rad$^+$ strain. After five crosses, the chromosomes of sibling isolates were analyzed by CHEF (CHU *et al.* 1986). For the *rad9-1* mutant, each of nine backcrossed siblings examined contained a chromosome *8* which was different in size from that of either the originally mutagenized strain (Java-6) or the backcross parent (six of the isolates are shown in Figure 1). CHEF analysis of the original *rad9-1* mutant showed that its chromosome *8* was identical in size to that of Java-6 (data not shown). In addition, the cloned *rad9* gene (ZOLAN *et al.* 1992) hybridized to this novel-sized chromosome *8* (ZOLAN *et al.* 1993). Thus, this novel-sized chromosome is a CLP marker for the location of the *rad9-1* mutation and appears to have been generated by the process of backcrossing.

To investigate whether CLP markers were present in other *rad* mutants, we analyzed sibling backcrossed isolates of *rad3-1* and *rad 11-1*. For *rad3-1*, these isolates contained a novel-size chromosome *13*, which remained unchanged through further backcrosses to Okayama-7 (Figure 2A). Two separate backcrosses were examined. For both, all of the Rad$^-$ progeny contained a novel-sized chromosome *13* identical to the one shown in Figure 2A. In a detailed examination of one of these backcrosses, 12 Rad$^-$ progeny were examined, and each had a chromosome *13* the size of the mutant parent's chromosome *13*. All eight of the examined Rad$^+$ progeny had a chromosome *13* the size of the wild-type parent's (a subset of these progeny is shown in Figure 2B). This segregation pattern indicated that the *rad3* gene is on chromosome *13*. To test this hypothesis, we constructed a chromosome-specific cosmid library of chromosome *13*

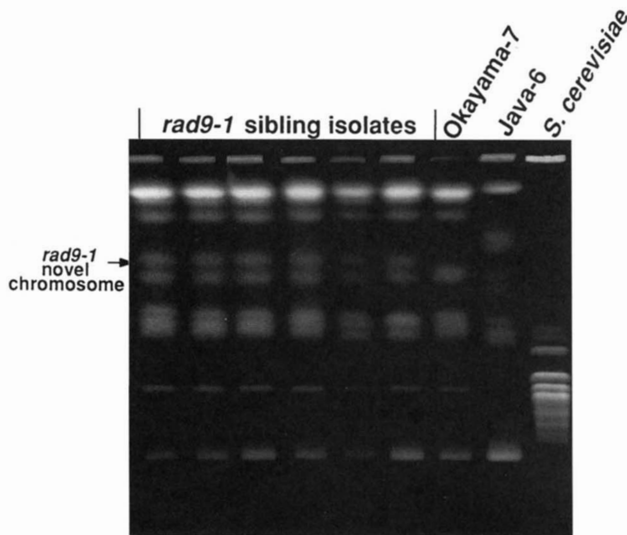

FIGURE 1.—Electrophoretic karyotypes of backcrossed *rad9-1* strains. A 1% agarose CHEF gel was run for 144 hr at 60 V, with a switch time of 22 min. The *rad9-1* isolates shown are sibling mutant isolates from the fifth generation of crossing and backcrossing of the *rad9-1* mutant (made in a Java-6 background) and Okayama-7. The arrow points to the novel-sized chromosome found in all sibling backcrossed *rad9-1* strains.

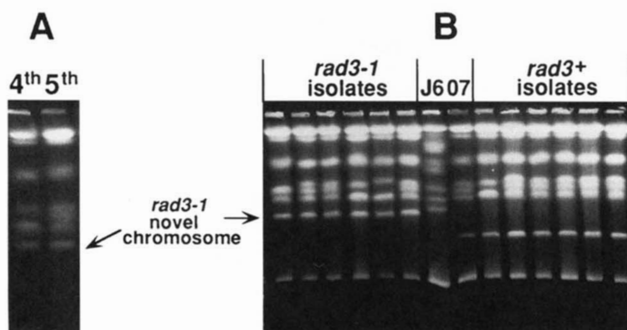

FIGURE 2.—Cosegregation of the *rad3-1* novel chromosome with the Rad⁻ phenotype. (A) Comparison of *rad3-1* isolates after four (4th) and five (5th) backcrosses to strain Okayama-7. B. The fourth generation *rad3-1* strain shown in (A) (3-1;4-29) was crossed to strain Okayama-7 (O7), and random basidiospore progeny were isolated, screened for radiation sensitivity, and analyzed electrophoretically. The strain Java-6 (J6) is shown for comparison. The CHEF gels were run using the following conditions: 0.9% agarose, 18 min pulse time, 60 V, 144 hr. The arrows point to the novel-sized chromosome found in all sibling backcrossed *rad3-1* strains.

from strain Okayama-7 (ZOLAN *et al.* 1992). RFLP analysis using two clones from this library revealed tight linkage between these clones and the *rad3* gene (Table 2), and both clones hybridized to chromosome *13* in Southern hybridizations of Chef blots. Therefore, these data confirmed that chromosome *13* is most likely the location of the *rad3* locus.

For *rad11-1*, RFLP analysis using a cloned copy of the *C. cinereus* ribosomal RNA gene (rDNA) cluster as a probe showed that the *rad11* gene is approximately 9 map units from the rDNA locus (Table 2). The rDNA gene cluster is known to be located close to the centromere of chromosome *6* (CASSIDY *et al.* 1984; PUKKILA and CASSELTON 1991). Analysis of the electrophoretic karyotypes of backcrossed strains (Figure 3A) indicated a novel-sized chromosome *6*, which was positively identified as the rDNA-containing chromosome by hybridization of the gel shown in Figure 3A with an rDNA probe (Figure 3B). As was the case for *rad9-1* (Figure 1) and *rad3-1* (Figure 2), all 12 sibling backcrossed *rad11-1* isolates examined contained a chromosome *6* that was intermediate in size between that of the original mutant (of Java-6 origin) and the backcross parent (Okayama-7).

TABLE 2

RFLP mapping of *rad* genes

Cross	Probe	Enzyme	Percent recombination	*N*
rad3-1 × MZC3	O7-13;1E4[a]	*Bam*HI	0	110
rad3-4 × MZC3	O7-13;1D5[a]	*Sac*I	1.7	116
rad11-1 × MZC3	pCc1[b]	*Bam*HI	9.4	117

[a] ZOLAN *et al.* (1992).
[b] WU *et al.* (1983).

Formation of CLP markers: Ultraviolet light is known to cause chromosomal changes in filamentous fungi (PERKINS 1974). Therefore, we investigated whether the initial mutagenesis of strain Java-6 had created the novel-sized chromosomes we observe in *rad* mutants. The analysis of original and backcrossed mutants for three different *rad3* alleles (Figure 4) showed that the mutagenic event was not responsible for the formation of CLP marker chromosomes in these strains. In each case, the original mutant has a chromosome *13* just like that of Java-6, and this size is stable through repeated subcultures and long storage at −80° (data not shown). In contrast, backcrossed isolates of the four examined *rad3* alleles contain a chromosome *13* slightly smaller than that of Java-6 (one isolate for each allele is shown in Figure 4). Therefore, a meiotic cross is necessary for the generation of these novel-sized chromosomes.

A model for the generation of these new chromosome sizes is meiotic recombination between homologs of different sizes (Figure 5). A cross between any strains with different length chromosomes could generate progeny with new chromosome sizes, as long as the new karyotypes were composed of viable combinations of genes. This model predicts that the formation of CLP marker chromosomes in our *rad* mutants is not related to the fact that these mutants are defective in DNA metabolism. To test this prediction, we examined the karyotypes of progeny of a cross between non-mutagenized Java-6 and Okayama-7, and observed numerous examples of new chromosome sizes in the progeny (data not shown). In addition, a similar phenomenon was observed when we backcrossed a *trp1* mutant strain to Okayama-7 (ZOLAN *et al.* 1993).

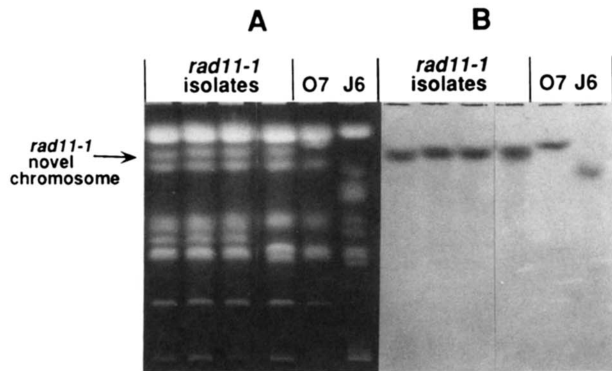

FIGURE 3.—Electrophoretic karyotypes of backcrossed *rad11-1* strains. (A) A 1% agarose CHEF gel was run at 60 V for 144 hr days with a pulse time of 22 min, followed by 24 hr with a pulse time of 60 min. (B) The gel was blotted and hybridized with a cloned copy of the *C. cinereus* ribosomal RNA genes (WU *et al.* 1983). The *rad11-1* isolates shown are sibling mutant isolates from the fifth generation of crossing and backcrossing of the *rad11-1* mutant (made in a Java-6 background) and Okayama-7. Abbreviations are the same as those used in Figure 2.

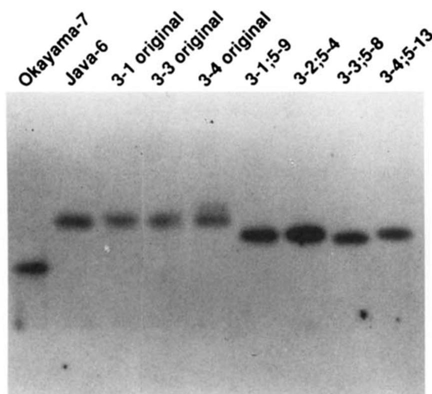

FIGURE 4.—Comparison of original and backcrossed *rad3* mutants. A 1% CHEF gel was run for 144 hr at 60 V, with a switch time of 22 min, then blotted and hybridized with a portion of the clone O7-13;1E4 (Table 2; ZOLAN *et al.* 1992). The chromosomes of the original *rad3-2* strain were too faint to reproduce; chromosome *13* was identical to those of the other original mutants.

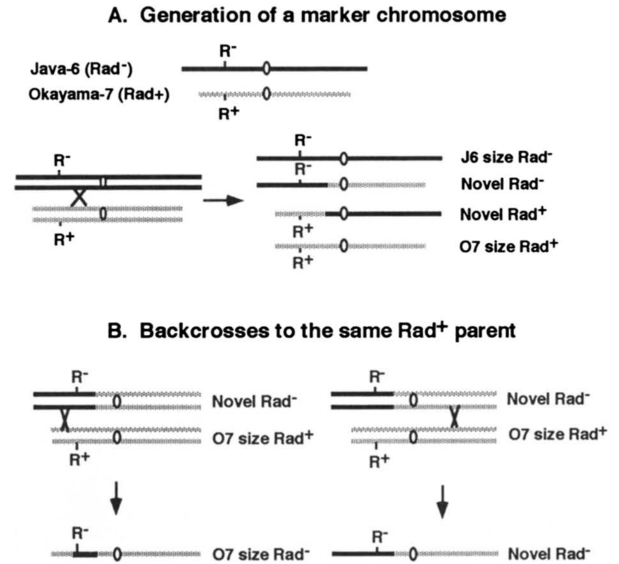

FIGURE 5.—Recombination model for the generation of CLP markers in backcrossed mutant strains. (A) Meiotic recombination between homologs of different lengths. (B) Further crosses of the recombinant chromosome to the same backcross parent.

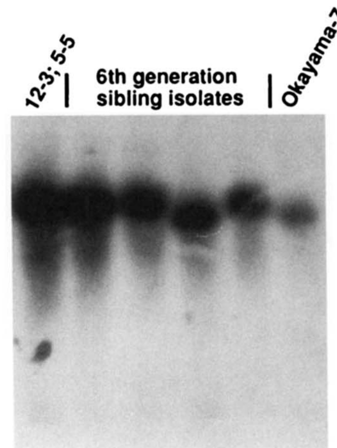

FIGURE 6.—Inheritance of the *rad12-3* marker chromosome through further backcrosses. Strain 12-3;5-5 was crossed to Okayama-7, and random basidiospore progeny were isolated, screened for radiation sensitivity, and analyzed electrophoretically. A 1% agarose CHEF gel was run under the same conditions as the gel shown in Figure 3, then blotted and hybridized with a portion of the cloned *rad9* gene (ZOLAN *et al.* 1992) which is 6.9 map units from the *rad12* gene (ZOLAN *et al.* 1993).

A further prediction of the model is that all mutant chromosomes will eventually become like that of the backcross parent, unless recombination is restricted between the mutational site and its telomere. As shown in Figure 5B, if recombination is between the mutational site and the telomere of that chromosome arm, then a mutant chromosome the size of the homolog of the wild-type parent will be produced. If recombination is on the other side of the mutation, the novel size of the mutant chromosome will be unchanged. A cross between a fifth generation backcross isolate of *rad12-3* and Okayama-7 yielded progeny that fulfill this prediction (four progeny of this cross are shown in Figure 6). Of eight mutant progeny examined, seven had a chromosome *8* the size of the *rad12-3* parent, indicating a crossover as shown on the right of Figure 5B. One progeny isolate had a chromosome *8* apparently the size of the Okayama-7 parent; this size was confirmed by running chromosome plugs of the progeny isolate and strain Okayama-7 side by side and within the same lane of another CHEF gel (data not shown).

Crosses between original mutants and Okayama-7: Most of our observations of CLP marker chromosomes were made after five or more rounds of backcrossing between a mutant strain and Okayama-7. By this stage,

TABLE 3

Size of mutant chromosomes in progeny of crosses between original mutants and Okayama-7

	Size			
Mutant	Java-6	Okayama-7	Other	*N*
rad3-1	4	1	0	5
rad3-4	2	0	3	5
rad9-1	4	1	0	5

as demonstrated for *rad9-1* (Figure 1), *rad3-1* (Figure 2), and *rad11-1* (Figure 3), most of the chromosomes of mutant strains are like the backcross parent, with the prominent exception being the mutant chromosome. To examine the initial generation of these mutant chromosomes of novel size, we looked at the first generation progeny of crosses between mutants and Okayama-7. The simple recombination model shown in Figure 5 predicts that both parental and marker size mutant chromosomes would be present in these progeny. However, the majority of strains recovered had the parental (Java-6) size mutant chromosome (Table 3; Figure 7). Of 15 first generation isolates examined, only one isolate, a *rad3–4*, had a mutant chromosome potentially of the size observed in fifth generation backcross isolates (comparison not shown). In addition, it is apparent from the *rad3-1* and *rad3-4* isolates that a range of new mutant chromosome sizes is formed, implying that recombination may be possible between the *rad3* mutant chromosome and more than one wild-type chromosome, or that more than one pairing configuration may be possible between mutant and wild-type homologs, or that insertion/deletion heterologies exist along the lengths of the chromosomes such that crossovers in different places yield differently sized chromosomes. Since the Java-6 chromosome *13* is almost twice the size of the Okayama-7 chromosome *13*, it is likely that sequences homologous to that Java-6 chromosome are found on more than one Okayama-7 chromosome. This prediction was confirmed by hybridization experiments: seven clones from a Java-6 chromosome *13* library were examined. Each hybridized to chromosome *13* of Java-6, but only five hybridized to chromosome *13* of Okayama-7; the other two hybridized to different Okayama-7 chromosomes (data not shown).

Crosses between backcrossed isolates and Okayama-7: To examine one pair of homologs in the absence of polymorphism between other chromosome pairs, we examined a cross between Okayama-7 and a *rad3-4* mutant (strain 3-4;4-1) which had been through four rounds of crossing to Okayama-7. In this *rad3-4* isolate, the *rad3* chromosome (chromosome *13*) is the size of the Java-6 progenitor of the strain. Random spore analysis of this cross (Figure 8A) showed that Rad⁻ progeny had the chromosome *13* sizes predicted by a recombination model for the generation of marker chromosomes: chromosome *13* in Rad⁻ isolates was the size of either the Rad⁻ parent, the Rad⁺ parent, or the CLP marker also found in *rad3-1* strains. However, tetrad analysis of the same cross (Figure 8B) showed that the chromosome *13* sizes observed in the random spore isolates cannot be explained by simple recombination between chromosome *13* of the two parental strains; in each of the three tetrads examined, only one chromosome *13* is non-parental in size, implying that recombination has occurred between chromosome *13* of the *rad3-4* strain and a different chromosome in strain Okayama-7; presumably the reciprocal product was unresolved in these gels.

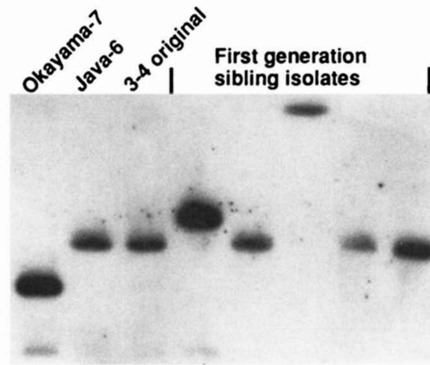

FIGURE 7.—The first outcross of *rad3-4* to Okayama-7. The original *rad3-4* isolate (in the Java-6 background) was crossed to Okayama-7, and random basidiospore progeny were isolated and screened for radiation sensitivity. Mutant progeny were then analyzed electrophoretically. A 1% agarose CHEF gel was run for 144 hr at 60 V, with a switch time of 22 min, then blotted and hybridized with a portion of the clone O7-13;1E4 (Table 2; ZOLAN *et al.* 1992).

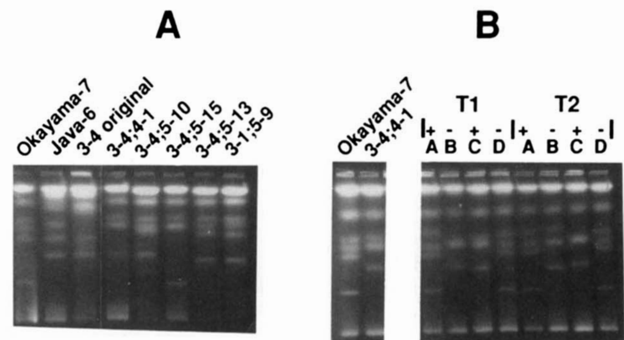

FIGURE 8.—Analysis of the *rad3-4* CLP marker in a further cross to Okayama-7. Strain *rad3-4;4-1* (3-4;4-1) was crossed to Okayama-7, and random basidiospore progeny (A) or tetrads (B) were isolated, screened for radiation sensitivity, and analyzed electrophoretically. In (A), only mutant isolates are shown, and a *rad3-1* isolate (3-1;5-8) is shown for comparison. In (B), (+) and (–) indicate a Rad⁺ or Rad⁻ phenotype of the isolate, respectively. For both (A) and (B), a 1% agarose CHEF gel was run for 144 hr at 60 V, with a switch time of 22 min.

Tetrad analysis of a cross between a backcrossed *rad3-1* mutant and Java-6. We had observed that the size of the mutant chromosome for a given *rad* mutant (*e.g.*, *rad9-1*, Figure 1; *rad3-1*, Figure 2) often stabilized in

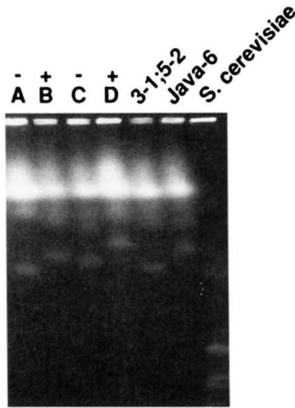

FIGURE 9.—Tetrad analysis of a cross between a backcrossed *rad3-1* isolate and Java-6. Strain *rad3-1;5-2* (3-1;5-2) was crossed to strain Java-6, and tetrads were isolated, screened for radiation sensitivity and analyzed electrophoretically. One tetrad is shown; (+) and (–) refer to the radiation phenotypes of the isolates. In order to maximize the resolution of chromosome *13*, a 1% agarose CHEF gel was run at 150 V for 72 hr, with the switch time linearly ramped from 1 to 5 min.

repeated crosses to the Okayama-7 parent. One explanation for this observation is that the novel-sized mutant chromosome, once generated, has common sequences with the homolog of Okayama-7 in only specific regions, thus restricting the sites of further recombination to one side of the *rad* gene. In these cases, recombination would not change the size of the mutant chromosome (Figure 5). In contrast, a cross between a backcrossed *rad* mutant and a different Rad$^+$ strain might be predicted to change the size of the mutant chromosome. We examined four tetrads of a cross between strain *rad3-1;5-2* and Java-6. In each tetrad, reciprocal recombination products were observed for chromosome *13* (an example is shown in Figure 9, see especially B and C), and new sizes for the mutant chromosome were observed.

DISCUSSION

We have observed that crosses between strains with polymorphic chromosomes yield progeny with new chromosome sizes and that repeated backcrossing of *rad* mutants to the same Rad$^+$ parent can result in a CLP marker, which cosegregates with the mutant phenotype. The segregation of these CLP markers has allowed us to predict the chromosomal locations of two genes for which no other mapping information was available: *rad3*, shown to be on chromosome *13* (Figure 2, Table 2), and *rad12*, shown to be on chromosome *8* (ZOLAN *et al.* 1993). In addition, the genes *rad9* (Figure 1) and *rad11* (Figure 3), for which mapping information was available, were shown to have CLP marker chromosomes as well. Thus, this analysis is useful for the assignment of unmapped genes to chromosomes and can be used to facilitate the chromosomal mapping of a new locus.

Several lines of evidence indicate that these new chromosomes are formed during meiosis, by recombination between chromosomes of different sizes. First, the mutant chromosome in non-outcrossed mutants is the same size as its wild-type homolog in Java-6 (data for *rad3* mutants are shown in Figure 4). Second, the electrophoretic karyotypes of the strains we have examined are stable mitotically. The only exception to this stability we have observed is the variability of chromosome *6*, which contains the rDNA locus, in strain Java-6 (data not shown). The size of the rDNA-containing chromosome has been shown to vary with the length of the entire rDNA repeat tract (as a result of changes in the number of rDNA repeats; PUKKILA and SKRZYNIA 1993), and a similar phenomenon has been reported for the protozoan parasite *Giardia lamblia* (ADAM 1992) Interestingly, we have not observed this rDNA chromosome variability in *rad* mutants, indicating that the *rad* genes we study may be involved in the intrachromosomal recombination of rDNA repeats.

We have also ruled out deletion of the original (Java-6) chromosome as a mechanism of formation of these new chromosome sizes, because the CLP marker chromosome can be larger than the original Java-6 chromosome (for example, see the *rad11* marker chromosome, Figure 3). In addition, crosses between nonpolymorphic strains do not produce progeny with new chromosome sizes (data not shown). Therefore, the novel-size mutant chromosomes we observe are most likely formed by some kind of interchromosomal recombination during meiosis. However, the simple model shown in Figure 5, although possibly correct for the formation of some marker chromosomes, probably cannot account for each case we have observed. For example, the frequency with which marker chromosomes are recovered is lower than that predicted from a simple recombination model. Only one chromosome recovered in random spore progeny of a first generation outcross appeared to be the size of a backcross CLP marker, even though the *rad* mutations segregated 1:1 in these crosses. Thus, there was no viability selection against mutant chromosomes. In addition, the stability we usually observe for a CLP marker means that recombination between the CLP marker and the homolog in the backcross parent is either inhibited completely or is restricted to one side of the gene, such that single crossovers do not change the mutant chromosome's size. Although some of the genes we have worked with may be telomeric, we know that *rad9* is more telomeric than *rad12* (ZOLAN *et al.* 1993) and yet the *rad9* CLP marker is stable while the *rad12-3* marker is not. In addition, an apparently stable CLP marker has been observed in backcrossed *trp1* isolates (ZOLAN *et al.* 1993), and the *trp1* gene is centromere-linked (NORTH 1987). We think it most likely that the stability of CLP markers is due to

ectopic events in their formation; a CLP marker is usually stable because it has homology to the homolog of the backcross parent in limited regions, and therefore recombination is restricted to one side of the gene. Hybridizations with random clones have revealed repeated sequences, present on multiple *C. cinereus* chromosomes (ZOLAN *et al.* 1992); in addition, hybridizations with single copy sequences have provided evidence for translocations. Therefore, in crosses between strains as karyotypically polymorphic as Java-6 and Okayama-7, multiple pairing and recombination partners are likely possible. What sorts out by the fourth or fifth backcross is most probably the reflection of the random assortment of viable karyotypes. In further backcrosses, ectopic recombination among dispersed repeats may be suppressed as the repeats become homozygous at allelic positions, as was demonstrated in *Drosophila melanogaster* (MONTGOMERY *et al.* 1991), in which ectopic recombination between dispersed repeats was shown to cause extensive chromosome rearrangement.

In the case of chromosome *13*, it is clear that sequences of this chromosome in Java-6 are located on more than one chromosome in Okayama-7, and the large strain difference in the size of chromosome *13* (1.8 Mb in Java-6 and 1 Mb in Okayama-7) reflects this fact, and may reflect a translocation such that two Okayama-7 chromosomes are homologs to chromosome *13* of Java-6. Our *rad3-1* segregation data (Figure 2) imply that the chromosomes *13* of Java-6 and Okayama-7 are indeed homologs, and a similarly great size difference in homologous chromosomes has been observed in wine strains of *Saccharomyces cerevisiae* (BIDENNE *et al.* 1992). However, although our RFLP data indicate that the *rad3* gene is tightly linked to sequences of the Okayama-7 chromosome *13* (Table 2) it is possible that this is pseudolinkage, and that the true location of the *rad3* gene is another chromosome involved in the translocation. The fact that all four of the *rad3* CLP markers are close in size (Figure 4) suggests that a limited number of alternative pairing partners is available to the Java-6 chromosome *13* in crosses to Okayama-7. In crosses of *rad3-1;5-2* to Java-6 (Figure 9), the *rad3-1* chromosome *13* and the Java-6 chromosome *13* are very close in size (about 1.7 Mb for the *rad3-1* strain and 1.8 Mb for Java-6) and are likely simple homologs in terms of their content, since this cross yields progeny tetrads with apparently reciprocal recombination products.

The nature of the CLP among *C. cinereus* strains is not known. Since a number of different electrophoretic karyotypes are viable, many of the length changes are likely of no genetic consequence. However, outcross tetrads have relatively low viability (PUKKILA 1992), indicating that at least some length changes represent translocations which must be balanced. The human pathogen *Plasmodium falciparum* has been shown to have extensive CLP, which maps to subtelomeric repeats present on some chromosomes, and highly variable subtelomeric repeats have also been observed in *S. cerevisiae* and *D. melanogaster* (reviewed in ZAKIAN 1989). It is possible that *C. cinereus* owes some amount of its chromosome polymorphism to repeat tracts of varying length, either telomeric or interstitial. However, it is not likely that such repeats could account for all of the length polymorphism we have observed, and ectopic recombination among dispersed repeats is likely to be involved in the generation of some of the CLP variability we observe.

The amount of new length polymorphism we observe after meiosis is greater than that reported for most organisms, although recently (PLUMMER and HOWLETT 1993) a similar amount was reported for the phytopathogenic fungus *Leptosphaeria maculans*. In addition, new chromosome sizes after meiotic crosses have also been reported for *P. falciparum* (WALLIKER *et al.* 1987), *S. cerevisiae* (ONO and ISHINO-ARAO 1988), and *Ustilago maydis* (KINSCHERF and LEONG 1988).

Our findings, and those of PLUMMER and HOWLETT (1993), run counter to the hypothesis of KISTLER and MIAO (1992) who suggested that changes in chromosome structure are neutral in the absence of meiosis and therefore asexual species would be more polymorphic than those with a sexual stage. PLUMMER and HOWLETT (1993) hypothesize that the meiotic variability of *L. maculans* allows it to adapt to new resistance genes in its host, oilseed rape, and a similar argument has been made for the mitotic variability of *Candida albicans* (RUSTCHENKO-BULGAC 1991) and for variability in the phytopathogen *Colletotrichum gloeosporioides* (MASEL *et al.* 1990). Our data show a large amount of chromosome length variation among interbreeding strains of *C. cinereus*, and it is unknown whether this variability is tolerated or exploited by the organism.

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