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Variability of karyotypes and RAPD types in genetically related strains of Cryptococcus neoformans — Source link

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ORIGINAL PAPER

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Variability of karyotypes and RAPD types in genetically related strains of *Cryptococcus neoformans*

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Abstract Variation in karyotypes and RAPD patterns of genetically related strains of *Cryptococcus neoformans* were analyzed. Capsular and filamentous mutants usually differ in their karyotypes from wild-types, but the RAPD patterns were found to be similar. Karyotype differences were observed in most heterothallic matings, but RAPD patterns remained identical. After self-sporulation of a diploid strain, minor chromosomal length polymorphism and minor changes in the RAPD types occurred. Three mechanisms, either alone or in combination, may in varying degrees contribute to the karyotype variation of *C. neoformans*: (1) mitotically induced changes; (2) karyotype changes as a result of meiotic recombination, and (3) mutagen-induced changes. The present data do not support the meiotic maintenance hypothesis, which claims that the amount of CLP generated is inversely proportional to the frequency of meiosis.

Key words *Cryptococcus neoformans* · Karyotype · RAPD patterns · Mating · Mutant

Introduction

Cryptococcus neoformans (Sanfelice) Vuillemin is a prominent medical yeast (Mitchell and Perfect 1995), which is found in nature in the asexual unicellular state. A sexual state can be obtained in vitro by pairing strains of opposite mating type, a or α (Kwon-Chung 1975, 1976, 1978). The usual heterothallic life cycle includes conjugation of haploid yeast cells, which results in dikaryotic hyphae with clamp connections, haustorioid branches, and cylindrical-clavate basidia. Following meiosis, four basipetal chains of haploid basidiospores are formed (Kwon-Chung 1975, 1976). In addition to the usual haploid cells, diploid yeast cells occur as well (White and Jacobson 1985; Whelan and Kwon-Chung 1986; Takeo et al. 1993). Self-sporulation (or monokaryotic fruiting), resulting in monokaryotic hyphae with incomplete clamp connections, has been reported from diploid strains with mating type αa (Erke 1976; Kwon-Chung 1978; Schmeding et al. 1981a; Whelan and Kwon-Chung 1986; Takeo et al. 1993), and from haploid wild-type strains with mating type α (Wickes et al. 1996). Additionally, the αa strains may show diploid-haploid mating, in which dikaryotic hyphae with clamp connections and basidia are formed (Schmeding et al. 1981a; T. Boekhout, unpublished observation). Substantial evidence indicates that natural propagation is mainly clonal (Brandt et al. 1995), which is in accordance with the occurrence of geographically restricted populations with similar karyotypes and RAPD patterns (Sorrell et al. 1996a, b; Boekhout et al. 1997).

In epidemiological studies of the species a considerable variation of karyotypes, DNA-fingerprints, and RAPD-genotypes has been demonstrated (Polacheck and Lebens 1989; Perfect et al. 1993; Dromer et al. 1994; Wickes et al. 1994; Brandt et al. 1995, 1996; Varma et al. 1995; Chen et al. 1996). The genetic make-up of the varieties *neoformans* and *gattii* was also found to differ from each other (Wickes et al. 1994; Varma et al. 1995; Boekhout et al. 1997). Karyotypes and RAPD patterns usually are stable within strains, but minor variations have been reported as well (Fries et al. 1996; Boekhout et al. 1997). This stability of karyotypes and other genetic markers within strains contrasts with a considerable inter-strain variability.

Given the apparent clonal propagation of the species, the question is how the overall variation of karyotypes and RAPD patterns is generated. We investigated karyotypes and RAPD patterns from the following groups of genetically related isolates: (1) capsular and filamentous mutants (Gordon and Devine 1970; Jacobson and Tingler 1994); (2) heterothallic matings (Kwon-Chung 1978; Schmeding et al. 1981b); and (3) the self-sporulating strain CBS 7816 with descendants (Schmeding et al. 1981a).

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Materials and methods

Genetically characterized strains of *C. neoformans* varieties *neoformans gattii* were obtained from the collections of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS, Delft, the Netherlands), the American Type Culture Collection (ATCC, Rockville, Maryland, USA), and Dr. E. S. Jacobson (Department of Veterans Affairs, Richmond, Virginia, USA) (Table 1). The identity of the strains was confirmed by testing colour reactions on CGB-medium, D-proline assimilation, serotyping, and killer-toxin sensitivity (Boekhout et al. 1997). Strains were maintained on 1% yeast extract, 0.5% peptone, 4% glucose agar (YPGA) slants at 10°C.

Table 1 Strains of *C. neoformans* employed. (Legends: ATCC = American Type Culture collection, Rockville, Maryland, USA; CBS = Centraalbureau voor Schimmelcultures, Baarn-Delft, The Netherlands; ESJ = E. S. Jacobson, Richmond, Virginia, USA; NIH = National Institutes of Health, Bethesda, Maryland, USA; RV = Institute of Tropical Medicine, Antwerp, Belgium; UCD = University of California, Davis, California, USA; cap = capsular mutant; mt = mating type; self = self-sporulating strain; NTG = N-methyl-N′-nitro-N-nitrosoguanidine; UV = ultra-violet)

CBS number	Other designation	Genetic background
6900	NIH B-3501	NIH 430×NIH 12, mt α
6901	NIH B-3502	NIH 430×NIH 12, mt a
7926	ESJ 59	cap59–; B-3501, mt α, NTG
7927	ESJ 55	cap55–; B-3501, mt α, NTG
7928	ESJ 60	cap60–; B-3501, mt α, UV
7929	ESJ 64	cap64–; B-3501, mt α, NTG
7930	ESJ 66	cap66–; B-3501, mt α, NTG
7931	ESJ 67	cap67–; B-3501, mt α, NTG
7932	ESJ 81	cap81–; B-3502, mt a, UV
7933	ESJ 195	cap+; B-3501, mt α
7934	ESJ 322	cap55–×B-3502, mt a
7935	ESJ 326	cap60–×B-3502, mt a
7936	ESJ 328	cap67–×B-3502, self
7937	ESJ 331	cap331–×B-3502, mt α
7938	ESJ 338	cap172h×B-3502, mt ?
7816	ATCC 34868 = NIH 371	Wild, cuckoo droppings, Thailand, self
7823	ATCC 42163	Single basidiospore 7816, mt α
7824	ATCC 42164	Single basidiospore 7816, mt a
7825	ATCC 42165	Single basidiospore 7816, self
7826	ATCC 42166	Single basidiospore 7816, self
7827	ATCC 42167	Single basidiospore 7816, self
6886	ATCC 28958 = NIH 430	Wild pigeon droppings, Denmark, mt a
7814	ATCC 32735 = RV 29192	Air, Belgium, mt α
7821	ATCC 42161	Single basidiospore 6886×7814
7822	ATCC 42162	Single basidiospore 6886×7814
7813	ATCC 28957 = NIH 12	Human bone lesion, mt α
6901	ATCC 34874	Single basidiospore 6886×7813
7817	ATCC 34873	Single basidiospore 6886×7813
7815	ATCC 34632	Pigeon droppings, Czechoslovakia
7000	ATCC 34875 = NIH 433	Pigeon droppings, Denmark
7828	ATCC 42168	Single basidiospore 7000×7815
7829	ATCC 42169	Single basidiospore 7000×7815
5728	UCD 67-57	Non-meningitic cryptococcal cellulitis and osteomyelitis, USA
5729	UCD 57-68	Hyphal mutant of 5728
7812	ATCC 24064 = NIH 68	Cerebrospinal fluid, USA
7820		chemically induced filamentous mutant of CBS 7812
6993	ATCC 24066 = NIH 18	Man, USA
7819	ATCC 36069	Chemically induced filamentous mutant of 7819

Pulsed-field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD) analysis were performed as described earlier (Boekhout et al. 1997). Densitometric analysis of the karyotypes was performed using a Bio-rad 620 densitometer and 1-D Analyst software (Biorad, Veenendaal, The Netherlands).

Results

Capsular and filamentous mutants

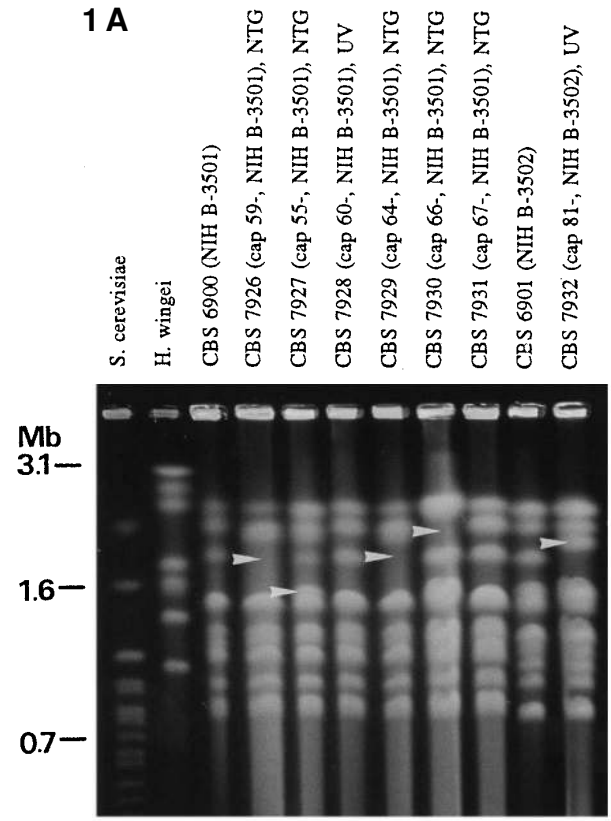
The sibling strains CBS 6900 (=NIH B-3501) and CBS 6901 (=NIH B-3502) (Kwon-Chung 1978) showed some chromosomal length polymorphism (CLP) (Fig. 1B, arrow heads). Karyotype variation was also found among UV- or N-methyl-N′-nitro-N-nitrosoguanidine (NTG)-induced capsular mutants of these two siblings (Jacobson and Tingler 1994) (Fig. 1, arrow heads), although the difference concerned only one or two bands. The second (approximately 2.2 Mb) and third (approximately 1.8 Mb) band from the top were most frequently affected by the mutagen. The mutant strains CBS 7926 (cap59–), CBS 7929 (cap64–), and CBS 7983 (cap+) differed from the parental strain NIH B-3501 by the absence of an approximately 1.8 Mb chromosome, CBS 7930 (cap66–) lacked an approximately 2.2 Mb-sized chromosome, and the NTG-mutant CBS 7927 (cap55–) possessed an additional chromosome of approximately 1.6 Mb (Fig. 1, arrow heads). The karyotypes of CBS 7928 (cap60–) and CBS 7931 (cap67–) were identical with the wild-type. The karyotype of CBS 7932, a UV-induced mutant of NIH B-3502, differed from its wild-type by the presence of a somewhat larger third chromosome of approximately 2 Mb (Fig. 1A, arrow head).

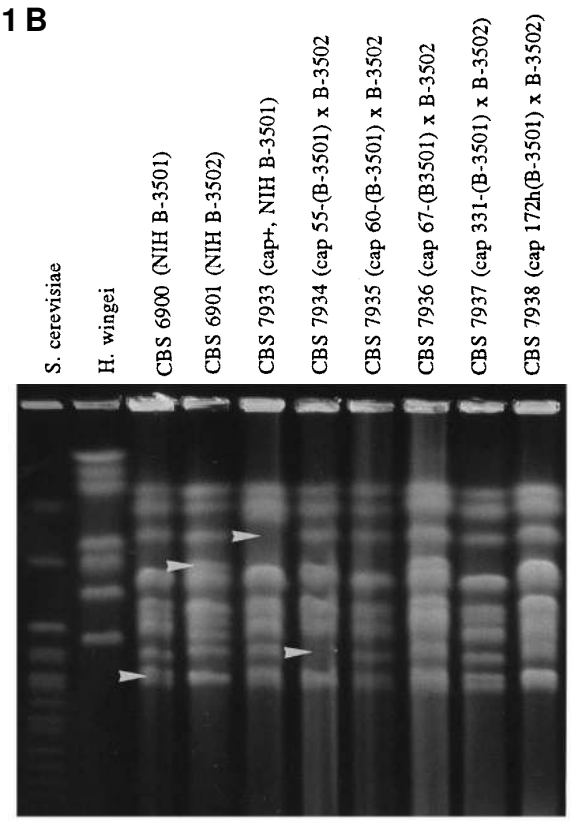
Chromosomes 1 and 4–11, according to the numbering of Wickes et al. (1994), were found to be stable after these mutagenic treatments. Crossings between some of the capsular mutants with NIH B-3502 (=CBS 6901), a sibling of the originally used wild-type strain NIH B-3501 (=CBS 6900) (Jacobson and Tingler 1994), may also cause changes in the karyotype (Fig. 1B). In most cases, karyotypes of the F_1 generation strains were identical with or similar to those of the F_0 generation, or else possessed a hybrid pattern. CBS 7934 (cap55–×NIH B-3502), however, lacked an approximately 0.9-Mb-sized chromosome (Fig. 1B).

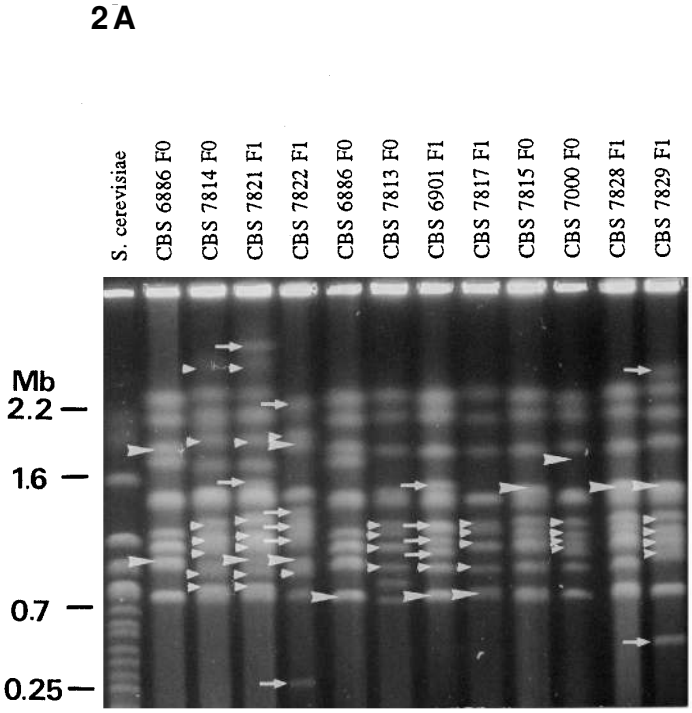
Karyotype differences were also observed when wild-type isolates CBS 5728 and CBS 7812 and their respective filamentous mutants CBS 5729 and CBS 7820 were compared (Fig. 2B). However, the karyotype of the filamentous mutant CBS 7819 was found to be identical with its wild-type strain CBS 6993 (data not shown). The RAPD patterns of the mutant strains were identical with those of the wild-types (data not shown).

Heterothallism

Karyotype changes were usually observed following mating (Fig. 2A, B), and comprised both inter- and intra-chro-







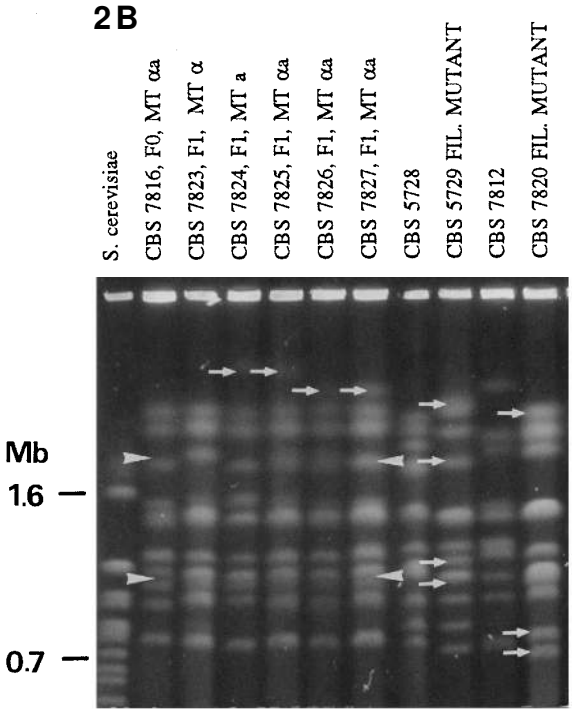


Fig. 1 A, B Karyotypes of capsular mutants of *C. neoformans* (for explanation of symbols see Table 1; *arrow heads* indicate the most significant changes in the karyotypes)

Fig. 2 A, B Karyotypes of heterothallic (panel *A*), self-sporulating and filamentous mutant strains (panel *B*) of *C. neoformans* (for explanation of symbols see Table 1; novel bands are indicated with arrows, and bands occurring in one of the parental strains are indicated by *small and large arrow heads*, respectively. In panel *B* chromosomes with CLP are indicated with a *large arrow head*)

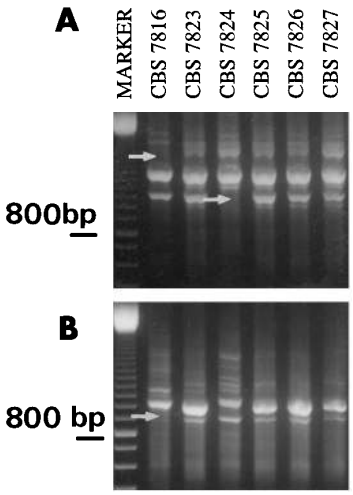


Fig. 3A, B RAPD pattern of the self-sporulating strain strain CBS 7816 and some of its F_1 offspring using ERIC1 (panel *A*) and ERIC2 (panel *B*) primers (*arrows* indicate changes in the RAPD types)

mosomal rearrangements, resulting in chromosomal length polymorphism (CLP). Intra-chromosomal changes included size variations, and rearrangements of partial or entire chromosomes were interpreted as inter-chromosomal changes.

The karyotype of CBS 7821, a F_1-generation isolate of the mating between CBS 7814 and 6886, had many bands in common with its parent strain CBS 7814 (small arrow heads). One band was obtained from the other parent strain (large arrow head), few bands occurred in both parents, and two unique bands of approximately 1.5 and >2.2 Mb sizes were present (arrows). The RAPD pattern of CBS 7821 was identical with that of CBS 7814. The karyotype of the sibling strain CBS 7822 (CBS 7814×CBS 6886) also possessed a hybrid pattern. Novel chromosomes of approximately 0.3 and 2.2 Mb were present, and the three chromosomes of approximately 1.0 Mb showed CLP (arrows). Two chromosomes of approximately 1.0 and 2.0 Mb were obtained from CBS 6886 (large arrow heads), and another of ≤2.0 and approximately 1.0 Mb were present in the other parent (small arrow heads). The RAPD pattern of CBS 7822 was similar to that of the parental strain CBS 6886.

The mating CBS 6886×CBS 7813 also resulted in karyotype changes (Fig. 2A). CBS 6901 possessed a unique band of approximately 1.6 Mb, and a series of bands of approximately 1.0 Mb showed CLP (arrows). The karyotype of the sibling strain CBS 7817 largely agreed with that of the parent strain CBS 7813 (small arrow heads), but the lowermost band occurred in parent strain CBS 6886 (large arrow head). The RAPD patterns of all these isolates were identical.

The karyotype of CBS 7828, a F_1-generation isolate from the mating CBS 7815×CBS 7000, was identical with that of parent strain CBS 7815. Its sibling CBS 7829, however, had a hybrid pattern with one band obtained from the parent CBS 7815 (large arrow head), four from CBS 7000 (small arrow heads), and two unique chromosomes occurred of >2.2 Mb and approximately 0.5 Mb (arrows).

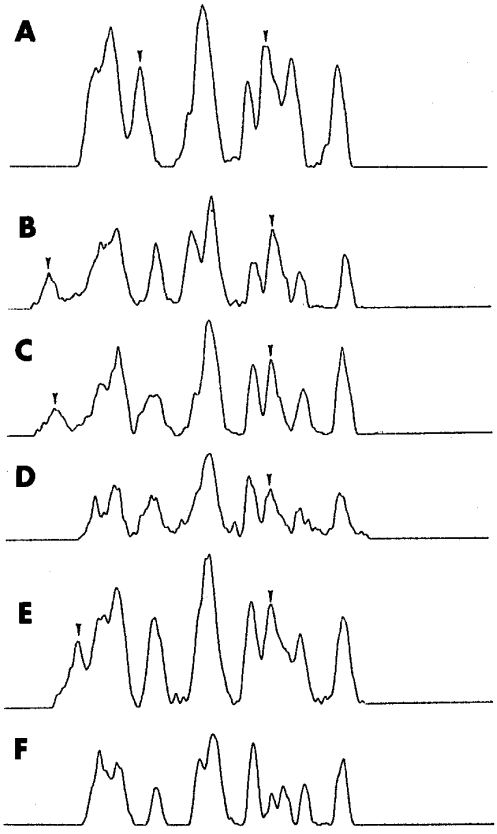


Fig. 4A–F Densitometric tracings of karyotypes of the self-sporulating strain CBS 7816 and some of its F_1 offspring. **A** CBS 7873, F_1, mt α; **B** CBS 7824, F_1, mt a; **C** CBS 7825, F_1, mt αa; **D** 7826, F_1, mt αa; **E** 7827, F_1, mt αa; **F** CBS 7816, F_0, mt αa. *Arrows* indicate novel chromosomes or altered densities

The RAPD patterns of these F_0 and F_1 generation strains were found to be identical.

Self-sporulation

Karyotypes of the F_1 generation isolates obtained from the self-sporulating strain CBS 7816 had nine bands in common with the parent strain (Fig. 2B). CLP was observed in the third band from the top (approximately 1.8 Mb), and in the two chromosomes of approximately 1.0 Mb (Fig. 2B large arrow heads; Fig. 4). Some F_1-strains, namely CBS 7824, 7825, 7826 and 7827, showed an additional large chromosome of >2.2 Mb (arrows). RAPD patterns of the F_0 and F_1 generations were rather similar, with the exception of the F_1 generation strain CBS 7824, which showed a somewhat different banding pattern with primers ERIC1 and ERIC2 (Fig. 3).

The largest chromosomes (≥2.2 Mb) were most frequently involved in karyotype modifications following heterothallic mating and self-sporulation. The chromosomes of approximately 2.2 Mb (chromosomes 1 and 2 of Wickes et al. 1994), approximately 1.5 Mb (chromosomes 4–6 of Wickes et al. 1994), and 0.7 Mb (chromosome 12 of Wickes et al. 1994) were relatively stable.

Discussion

Chromosome-length polymorphism (CLP) occurs in many species of yeasts and filamentous fungi (Zolan 1995), and this feature may be useful in epidemiological studies of plant-, animal- and human-pathogens (e.g. Cooley and Caten 1991; Perfect et al. 1993; Dromer et al. 1994). Our results indicate that karyotypes are more prone to variation than RAPD-types. A different RAPD pattern is usually reflected in a different karyotype, but differences in karyotypes do not always coincide with RAPD differences.

A better understanding of the mechanism of karyotype diversification in *C. neoformans* may be relevant to the analysis of variations observed in epidemiological studies (Fries et al. 1996; Boekhout et al. 1997), and to fungal genetics in general. An interesting paradox has been observed in *C. neoformans*, as well as in many other species, namely mitotic stability of karyotypes, i.e. low levels or absence of intra-strain variability, together with a considerable inter-strain variability (Perfect et al. 1993; Mitchell and Perfect 1995; Zolan 1995; Boekhout et al. 1997). Karyotypes of many species studied do not change during mitosis (Boekhout et al. 1991 a; Cooley and Caten 1991; Russell and Mills 1993; Talbot et al. 1993; Boehm et al. 1994), whereas meiosis usually results in changes of karyotypes (Ono and Ishino-Arao 1988; Plummer and Howlett 1993; Zolan et al. 1994; Martin 1995). The present and earlier studies (Perfect et al. 1993) suggested that karyotypes of *C. neoformans* are relatively stable mitotically, but that they can change considerably during meiosis. However, minor karyotype changes have been observed in a number of sequential isolates from one patient, and also after experimental infection of mice (Fries et al. 1996; Boekhout et al. 1997).

The observed combination of inter-strain variability, mitotic stability and meiotic instability of karyotypes in *C. neoformans* is not easy to explain. Linkage disequilibrium studies suggested that *C. neoformans* mainly propagates clonally (Brandt et al. 1995). If this is true, it seems unlikely that meiotic recombination contributes significantly to the observed inter-strain variability in nature.

Initially we did not conceive a major role for mitotic recombination in the process of generating karyotype variation in *C. neoformans.* However, an accumulation of small chromosomal changes may alter karyotypes after many generations (Zolan 1995). A number of observations support the occurrence of mitotic changes of karyotypes in *C. neoformans*: (1) CLP of the largest chromosome was observed in the *C. neoformans* var. *gattii* when using different storage regimes (Boekhout et al. 1997); (2) minor karyotype differences were observed in multiple isolates from a patient, as well as after passage through mice with clinical and saprobic isolates (Fries et al. 1996; Boekhout et al. 1997); (3) chromosome variation of *C. neoformans* was found to be induced by mutagenic treatments. Rapid mitotic change of karyotypes, as occurs in some asexual yeasts, e.g. *Candida albicans* (Rustchenko-Bulgac and Howard 1993), does not occur in *C. neoformans*. Karyotype changes, as seen in the hyphal mutants of *C. neoformans*, have also been observed after yeast-hyphal transitions in other species (Boekhout et al. 1991 b; McEachern and Hicks 1991), and after transformation experiments (Xuei and Skatrud 1994).

Karyotype variation of *C. neoformans* clearly increased after meiosis. Most of the chromosomes of the F_1 progeny could be traced to one or both of the parental strains using band size as a parameter. Several chromosomes were transferred without apparent length changes, whereas others showed length variation. Reciprocal recombination between homologous chromosomes of unequal size was suggested as an explanation of karyotype variation in *Leptosphaeria maculans* (Plummer and Howlett 1993, 1995). This model may also explain the occurrence of chromosomal reorganization after meiotic recombination in *C. neoformans*. The importance of this observation for the biology of the species is not clear, as sexual reproduction of the species has not yet been observed in nature. However, the occurrence of genetic recombination among populations of *C. neoformans* in nature cannot be ruled out completely, as was shown recently also for the asexual fungus *Coccidioides immitis* (Burt et al. 1996), and deserves further study.

C. neoformans possesses a number of alternative sexual strategies, such as heterothallism, self-sporulation of haploid and diploid isolates, and mono-dikaryotic matings (Kwon-Chung 1978; Schmeding et al. 1981 a, b; Takeo et al. 1993; Wickes et al. 1996), which may affect the chromosomal make-up in various ways. The importance of these different sexual strategies is not yet clear, as they have thus far been observed only in the laboratory. Self-sporulation of a diploid strain of *C. neoformans* resulted in relatively minor intra-chromosomal changes, whereas more extensive changes occurred following heterothallic matings. This suggests that different mechanisms of chromosomal transfer operate during self-sporulation and heterothallism. Mutagen-sensitive sites may be unequally distributed among the genome, as certain chromosomes of *C. neoformans* were found to be more sensitive to mutagenic treatment than others. Karyotype variation in *C. neoformans* appears to be genetically neutral as thus far no obvious correlation has been observed with any beneficial phenotypic changes. RAPD patterns were usually not altered after meiotic recombination or mutagenic treatments, but minor changes occurred in the RAPD patterns of F_1 isolates following self-sporulation.

Our results on *C. neoformans* (see also Boekhout et al. 1997) do not support the meiotic-maintenance hypothesis, which claims that the amount and extent of karyotype variation is inversely related with the frequency of meiosis (Kistler and Miao 1992). According to our present knowledge, three independently acting mechanisms may contribute in different degrees to the observed karyotype variation in *C. neoformans*: (1) mitotically induced changes; (2) mutagen-induced changes; and (3) meiotically induced changes. Further studies are required to analyze the relative importance of these three alternatives for the genetic make-up of the species, either in nature and in the laboratory. More detailed in-

sight into the micro-evolution of the genome of *C. neoformans* will contribute to our understanding of the population genetics and adaptation to the human host of this species.

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