Evidence for a more recently evolved clade within a *Candida albicans* North American population

Timothy J. Lott and Mohammad M. Effat†

Author for correspondence: Timothy J. Lott. Tel: +1 404 639 2459. Fax: +1 404 639 3546.
e-mail: tjll@cdc.gov

Mycotic Diseases Branch,
Division of Bacterial and
Mycotic Diseases, National
Center for Infectious
Diseases, Centers for
Disease Control and
Prevention, 1600 Clifton
Road, Atlanta, GA 30333,
USA

***Candida albicans* is diploid and displays a primarily clonal mode of reproduction. There is, however, evidence for meiosis and the degree to which this occurs in nature is unknown. Although random mating would act to obscure clonal lineages, previous studies have demonstrated that collections of North American isolates display three major partitions with no evidence of geographic clustering. To better understand the extent of sexuality and its role in the phylogeny of the species, a reference subset of 50 isolates representing this tripartite division was analysed using 1 minisatellite, 5 microsatellites (MSs) and 15 nuclear polymorphisms (NP). A total of 87 alleles were observed for 21 loci and 12/16 informative loci exhibited a departure from Hardy–Weinberg expectations ($G^2 \leq 0.05$). We did not observe an absolute correlation between MSs and NP, although isolates with identical NP genotypes were correlated with a previously defined, predominant class (putative group I). The use of additional markers did not give increased support for the tripartite structure of the population. However, (9/19) group I isolates were found to be highly related, differing by only one or a few alleles. Designated subgroup A, the interpretation is that these isolates are related by descent and that they are of a more recent evolutionary origin, diverging from an ancestral group I clone. The reason for their relative abundance in the population is unknown; one possibility is that they may be under positive selection.**

Keywords: genetics, population structure, SNPs, microsatellites

INTRODUCTION

The commensal yeast, *Candida albicans*, is a ubiquitous component of the normal human microflora. In non-immunocompromised individuals this species is often the agent of superficial epithelial infections. However, if normal immunity is reduced, *C. albicans* is capable of causing severe invasive disease. In the United States there has been an increase in immunosuppressed patient populations (Pfaller, 1995; Klein *et al.*, 1984; Johnson *et al.*, 1995). It is not surprising, therefore, that the number of *C. albicans* infections has been increasing and at present it is the eighth most common nosocomial bloodstream infection (Gerberding *et al.*, 1999).

This increase in infection rate has resulted in a renewed interest in the genetics and epidemiology of this organism. *C. albicans* is diploid, with a haploid chromosomal number (N) of eight (Chu *et al.*, 1993). An analysis of chromosomal stability has shown that the species undergoes relatively high rates of non-homologous mitotic recombination, translocation and aneuploidy (Thrash-Bingham & Gorman, 1992; Rustchenko-Bulgac, 1991). Although originally considered to reproduce as a clonal population (due to diploidy and the lack of a known sexual cycle), several initial analyses concluded that Hardy–Weinberg equilibrium could not be ruled out for some loci (Pujol *et al.*, 1993; Graser *et al.*, 1996). Additional work has yielded a consensus that *C. albicans* is primarily clonal, but with limited recombination (Forche *et al.*, 1999). However the extent of sexuality in nature remains unclear. For example, a high degree of mitotic recombination/

† Present address: National Research Center, Cairo, Egypt.

Abbreviations: MS, microsatellite; NP, nuclear polymorphism.

Table 1. Summary statistics for *C. albicans* isolates

A, ALS1; B, ERK1; C, CEF3; D, A3; E, A4; H, ZNF1; I, IS1; J, F16N1.3; K, F16N1.4; L, F16N1.5; M, F16N1.6; N, E15N6.1; O, F12N4.1; P, F16N1.1; Q, F16N1.2; R, E15N7.1; S, E15N7.3; T, C18N1.1; U, C20N4.1; V, E19N1.1; W, C2N8.1. ND, Not determined.

	A	B	C	D	E	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
Alleles	21	9	13	5	4	10	2	2	1	2	1	2	2	2	1	1	1	2	2	2	2
Ne	8.46	3.31	6.37	3.23	2.47	2.62	1.67	1.62	1	1.06	1	1.08	1.81	1.1	1	1	1	1.83	1.41	1.57	1.92
χ^2	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	ND	< 0.001	ND	< 0.001	< 0.001	0.74	ND	ND	ND	< 0.001	0.59	< 0.001	< 0.001
G ²	0.99	0.11	< 0.001	0.01	< 0.001	0.06	< 0.001	< 0.001	ND	0.009	ND	< 0.001	< 0.001	0.64	ND	ND	ND	< 0.001	0.58	< 0.001	< 0.001
L ₉₅ *	0.07	0.16	0.12	0.25	0.31	0.15	0.50	0.50	ND	0.50	ND	0.50	0.50	0.50	ND	ND	ND	0.50	0.50	0.50	0.50

* Calculated using 1000 simulated samples.

aneuploidy would tend to reduce the number of heterozygotes in the population, thereby driving a departure from Hardy–Weinberg equilibrium. In addition the lack of phylogenetic constructs that are statistically supported (Tibayrenc, 1997; Vilgalys *et al.*, 1997) is not expected for a primarily clonal organism. Finally, the recent discovery of a mating-type locus and its manipulation *in vitro* to allow cells of opposite ‘type’ to fuse, forming tetraploids, would argue either for a cryptic sexual cycle or one that has been active in the evolutionary history of the species (Hull *et al.*, 2000; Magee & Magee, 2000).

Despite the obscurity of clonality by meiosis, several studies of *C. albicans* population structure using phylogenetic approaches have demonstrated a tripartite division of non-epidemiologically related isolates. Pujol *et al.* (1997) showed parity between multilocus enzyme electrophoresis (MLEE), randomly amplified polymorphic DNA (RAPD) and Southern blot fingerprinting using a middle-repetitive probe. For these three groups, designated I–III, the PEP3^A allele was observed to be synapomorphic with group II (Pujol *et al.*, 1997). This tripartate structure has been supported by other work, using different populations and methodologies (Schmid *et al.*, 1999; McCullough *et al.*, 1995), although it has not been universally observed (Xu *et al.*, 1999a). Recently, we have observed this division in a North American population using alleles at loci ALS1 (Hoyer *et al.*, 1995), CEF3 (Bretagne *et al.*, 1997), ERK1 (Field *et al.*, 1996) and the nuclear insertion element IS1 (Mercure *et al.*, 1993). Moreover, we established that the presence of the IS1 element is highly correlated with group III and, through inference, that this is the oldest of the three lineages (Lott *et al.*, 1999).

In a recent study, Xu *et al.* (1999b) uncovered 15 anonymous single nuclear polymorphisms (NPs) which acted as independent codominant markers in their defined population. Interestingly, approximately 40% of the non-epidemiologically related strains were of a common genotype (Xu *et al.*, 1999b). Consequently, we were interested in whether these additional loci would suggest that recombination is occurring within a subset of our previously described population of North American bloodstream isolates (Lott *et al.*, 1999; Kao *et al.*, 1999), and in determining the degree of correlation

between microsatellites (MSs) and NPs. If found to be correlated, we were interested in whether these loci would give added support for clonality through a coalescence approach. In addition, we also report here on the finding of three new anonymous polymorphic MS markers, two of which were used in a population and phylogenetic analysis for a total of 21 loci.

METHODS

Isolates. From the 114 isolates previously described (Lott *et al.*, 1999), 50 were randomly chosen for additional analysis.

Genetic analysis. MS ZNF1 was analysed using a previously described primer set (Field *et al.*, 1996). Anonymous loci were found by searching the *C. albicans* genome database (sequence data for *C. albicans* was obtained from the Stanford DNA Sequencing and Technology Center website at <http://www-sequence.stanford.edu/group/candida>) for trinucleotide repeats of at least 6 (but less than approx. 25) repeat units in length.

Primers (20-mers) were based on non-repeated flanking regions giving predicted amplicons of between 100 and 300 bp. Forward primers were fluorescently 5′-end-labelled with either FAM or TET. PCR products were diluted in H₂O, denatured and analysed on an automated sequencer (ABI310) using GENESCAN software (Applied Biosystems). Anonymous loci primers were as follows. Locus A3, 5′-CAAGCTTATAGT-GGCTACTA-3′ (F), 5′-CCAACACTAGATACATCTCG-3′ (R); Locus A4, 5′-GTAATGATTACGGCAATGAC-3′ (F), 5′-AGAACGACGTGTACTATTGG-3′ (R); Locus A5, 5′-TAGTTCCTATTAGTAGTCAA-3′ (F), 5′-CAGGACTCC-AGTGCCCGTG-3′ (R). Locus A3 includes a nine-repeat unit of the trinucleotide TAA. Locus A4 includes 6 GAA repeats and locus A5 is a compound MS of 8 CAG repeats followed by a spacer and additional 5 and 4 tracts of CAG with a small intervening spacer. We observed that 8/50 strains exhibited four alleles at this locus and concluded that this region has been duplicated in these strains.

Primers and nomenclature for NP loci follow that of Xu *et al.* (1999b). *Taq* polymerase, dNTPs and buffers were from Boehringer Mannheim. Reaction conditions were as previously described (Lott *et al.*, 1999). All PCR reactions were carried out in 50 µl reactions with 35 cycles (following an initial denaturation for 5 min at 95 °C) of 1 min at 95 °C, 1 min at 55 °C and 1 min at 72 °C, followed by 5 min at 72 °C for final elongation. For NP loci the PCR products were ethanol-precipitated, resuspended in 20 µl H₂O and electrophoresed on 1.5% agarose. Gels were stained with ethidium bromide and photographed.

Genetic analysis was performed using Arlequin 1.1 (Schneider *et al.*, 1997) and Popgen 1.31 (Yang & Yeh, 1993). Phylogenetic analysis was performed using PHYLIP 3.5c (Felsenstein, 1992) and PAUP 4.0b (Swofford, 1998). For MS versus NP analysis, locus IS1 was grouped with the NPs. Similarity matrices were calculated as follows. For every given locus, if two isolates shared both alleles they were assigned a score of 1.0. If one allele was shared the value of 0.5 was assigned and for no common alleles a score of 0.0 was assigned. The summation was then divided by the total number of loci.

RESULTS

Population analysis

The summary statistics for the subset of 50 epidemiologically unrelated isolates are shown in Table 1. Loci ALS1, ERK1, CEF3 and IS1 have been described previously (Lott *et al.*, 1999). The ALS1 gene encodes a 106 bp minisatellite; ERK1 and CEF3 are MSs, and IS1 is an optional insertion element within the 25S rRNA (Mercure *et al.*, 1993). Locus zinc finger 1 is designated ZNF1 (Field *et al.*, 1996). Anonymous loci 3 and 4 are trinucleotide repeats and, similar to the other MS alleles, are given as the number of base pairs of the amplified PCR product. For anonymous locus 5, in some strains there were four observed alleles per strain. We conclude that this gene has been duplicated in these strains and that the paralogue has conserved primer sites. There were 14 observed alleles at this locus, yet due to the duplication it was not included in the population and phylogenetic analysis. For loci F16N1.3–C2N8.1 the nomenclature for homo- and heterozygosity follows that of Xu *et al.* (1999b). The total number of observed alleles for each locus is given, followed by the effective allele number, denoted N_e (Schneider *et al.*, 1997). This represents the number of equally frequent alleles that would be required to produce the same degree of homozygosity as observed in the population. We found that 5 of the 21 loci used in the population analysis were monomorphic, resulting in 16 informative loci. For the MSs we consistently observed that 1–3 alleles were predominant in the population, with lower frequencies for the remainder. The allelic sum was 87 and the mean N_e was 2.59 ± 2.0 . The probability of Hardy–Weinberg equilibrium based on χ^2 (Hartl & Clark, 1997) is given in Table 1. Fifteen of 16 (93%) loci were observed to have $P \leq 0.05$. During this analysis we observed that in some occurrences $|o - e| > e$, where o is observed frequency and e is expected frequency). We therefore calculated the likelihood ratio test (G^2) probability values (Yang & Yeh, 1993), which are given in Table 1. Under these criteria, 12/16 (75%) loci had $P \leq 0.05$. We conclude, therefore, that between 75 and 90% of the loci exhibited a significant departure from Hardy–Weinberg expectations. When all pairwise combinations of loci were tested for linkage disequilibrium (Schneider *et al.*, 1997), none of the 120 P values exceeded 0.05 (data not shown). This result suggests a clonal population structure.

We then calculated the probability of neutrality for each locus, using the Evans–Watterson test (Yang & Yeh,

Fig. 1. UPGMA phenogram (unrooted) for 21 loci. The box denotes isolates belonging to subgroup A. Horizontal axis, similarity coefficient. Asterisks denote isolates previously identified as group I and open boxes indicate isolates of group III; the remainder are group II (Lott *et al.*, 1999).

1993). Under the assumption of neutrality, the upper and lower 95% confidence limits were calculated and the lower (L_{95}) values are given in Table 1. In no case

Table 2. Individual *C. albicans* genotypes for members of subgroup A

Letters for corresponding alleles are as described in Table 1. F, A5; G, A5.2 (this locus was absent in these strains). The complete database is available electronically upon request. Nu., Isolate number; Loc., location (GA, Georgia; SF, San Francisco). Bold numbers indicate alleles differing from the majority consensus.

Nu.*	Loc.†	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
92-106	GA	16,16	231,239	126,135	132,141	117,120	178,178	0,0	145,168	0,0	2\2	1\1	2\2	1\1	1\1	1\2	2\2	2\2	2\2	2\2	2\2	2\2	1\1	2\2
92-156	SF	15,16	231,239	126,135	132,141	117,120	178,216	0,0	145,168	0,0	2\2	1\1	2\2	1\1	1\1	1\2	2\2	2\2	2\2	2\2	2\2	2\2	1\1	2\2
92-272	GA	16,16	231,239	126,135	132,141	117,117	178,216	0,0	145,168	0,0	2\2	1\1	2\2	1\1	1\1	1\2	1\2	2\2	2\2	2\2	2\2	2\2	1\1	2\2
92-105	GA	16,19	231,239	126,135	132,138	117,120	178,216	0,0	145,168	0,0	2\2	1\1	2\2	1\1	1\1	1\2	2\2	2\2	2\2	2\2	2\2	2\2	1\1	2\2
92-376	SF	11,16	231,239	126,135	132,141	117,120	178,178	0,0	145,168	0,0	2\2	1\1	2\2	1\1	1\1	1\2	2\2	2\2	2\2	2\2	2\2	2\2	1\1	2\2
92-379	SF	15,16	231,239	126,135	132,141	117,120	178,216	0,0	145,168	0,0	2\2	1\1	2\2	1\1	1\1	1\2	2\2	2\2	2\2	2\2	2\2	2\2	1\1	1\1
92-402	GA	16,19	231,239	126,135	132,141	117,120	178,216	0,0	145,168	0,0	2\2	1\1	2\2	1\1	1\1	1\2	1\2	2\2	2\2	2\2	2\2	2\2	1\1	2\2
93-208	GA	16,16	231,239	126,135	132,141	117,120	178,216	0,0	145,168	0,0	2\2	1\1	2\2	1\1	1\1	1\2	2\2	2\2	2\2	2\2	2\2	2\2	1\1	2\2
93-731	GA	15,16	231,239	126,135	132,141	117,120	178,216	0,0	145,168	0,0	2\2	1\1	2\2	1\1	1\1	1\2	2\2	2\2	2\2	2\2	2\2	2\2	1\1	1\2

was $P \leq 0.05$ (i.e. beneath the lower limit for neutrality), although for ALS1 the L_{95} was 0.07. Therefore, we conclude that, with the possible exception of ALS1, each locus was neutral.

Phylogenetic analysis

A pairwise distance matrix was generated where each locus was given equal weight. From this, a phenogram was constructed using an unweighted pair group analysis (UPGMA) with arbitrary rooting and is shown in Fig. 1. The mean similarity equalled $69.5 \pm 11.5\%$. Isolates previously identified as belonging to putative groups I, II and III (Lott *et al.*, 1999) are shown to the right of the phenogram. Most group I isolates clustered as a unit and, within these, we observed a subset of more highly related isolates termed subgroup A. The complete genotypes of these nine strains are given in Table 2. Although no two strains in the 50 analysed had identical genotypes, these nine isolates differed by only one to a few alleles. As seen in Fig. 1, strains previously identified as belonging in groups I and III did tend to cluster, although there was some intermixing of the two groups. An analysis of the data using parsimony (Swofford, 1998) with heuristic searching methods was used to compare trees with those generated by UPGMA (data not shown). A bootstrap analysis failed to produce statistical evidence for a specific topology ($P \geq 70\%$). However, what was uniformly observed in the majority consensus trees was the grouping of nine highly related strains. This was also found in UPGMA analysis, as seen in Fig. 1.

To determine the extent of parity between MS and NP markers, the two datasets were analysed separately using both parsimony and distance-matrix methods. The same general conclusions were found as when the data were pooled; there were no trees observed with more than one or two minor branches with bootstrap values above 70%. The UPGMA phenograms for MSs and NPs (including locus IS1) are shown in Fig. 2. Absolute parity between the two marker sets was not observed; however, neither was there total discordance.

Significantly, subgroup A, that was observed to be related by MSs, was associated with two groups of NPs. As seen in Fig. 2 and Table 2, five isolates of subgroup A had a common NP genotype, while four isolates differed at loci P (F16N1.1) and W (C2N8.1), thus placing them in a separate NP clade.

DISCUSSION

Clonality and recombination in *C. albicans*

Our analysis of this collection of North American isolates lends additional support that clonality plays a major role in the genetics of *C. albicans* (Pujol *et al.*, 1993; Forche *et al.*, 1999). For 15 bi-allelic loci analysed, five were found to be of a single type. This was not unexpected in that Xu *et al.* (1999b) observed low frequency of polymorphisms at these loci, with the less frequent allele ranging from approximately 0.01 to 0.04%. Overall, the allelic frequencies for the remaining 10 NPs were in accordance with those previously described. While Xu *et al.* (1999b) observed 5/9 of these loci (not including IS1) to be in Hardy-Weinberg equilibrium, we observed 2/9 NPs based on either χ^2 or G^2 . The collection of isolates analysed in the present study represents a surveillance set of epidemiologically unrelated (i.e. one isolate per patient) strains from several different hospitals. Therefore, we did not elect to use clone correction in analysing allelic frequencies (Xu *et al.*, 1999a). In addition, based on 23 loci (including A5 and its duplicated paralogue), no two isolates had an identical genotype.

Population structure: homoplasmy and relatedness by descent

In an analysis of 204 isolates using 15 NP markers, Xu *et al.* (1999b) observed that almost half (92/204) were of the same genotype. Using 14 of these NPs and IS1, we found that 24% (12/50) were of this same type, confirming this observation. Significantly, as shown in Fig. 2 (upper right-hand phenogram) this group is positively correlated with group I isolates (Fig. 2, upper-

Fig. 2. UPGMA phenograms (unrooted) for MSs and NPs. Isolates of identical NP genotype are boxed. Correlates for subgroup A isolates are shown by connecting lines. Asterisks denote isolates previously identified as group I and open boxes indicate group III; the remainder are group II (Lott *et al.*, 1999).

left phenogram). Furthermore, as seen in Fig. 1 and Table 2, within the group I isolates there is a clade of highly similar strains. Although it could be argued that selection is driving the formation of these genotypes, we believe that these findings present strong evidence that members of subgroup A are related by descent and together with the arguments presented above, can be viewed as clonally related. Thus we propose that the progenitor strain giving rise to subgroup A was a member of group I. As stated in our previous work (Lott *et al.*, 1999), our evidence suggests that groups I and II are derived from an older group III. Thus the creation of clade A would be of a more recent evolutionary origin

relative to the formation of the three major groups. Interestingly, as seen in Fig. 2 and Table 2, members of clade A can be divided into two groups depending on polymorphisms at two loci: F16N1.1 and C2N8.1. Both are Cfo1 polymorphisms and it is not known if, and how, they are related. It would logically follow, however, that these mutations were created following the formation of subgroup A. At present it is not known why members of subgroup A are relatively abundant (9/50 in the present population). One hypothesis is that they may be under positive selection. Since *C. albicans* is an obligate commensal, some form of host-mediated response would appear likely.

In this study we have observed that for at least some MSs, population structure can be correlated with NP mutations. This implies that they are not undergoing relatively higher rates of mutation; if so there would be no expected parity between marker sets. Rather, our interpretation is that neither MSs nor NPs alone may represent the true phylogenetic relationship between isolates and that this may be due to inherent limitations in each set. For MSs, it is known in *S. cerevisiae* and other species that large variations in mutation rates are observed depending on the type and length of repeat (Miret *et al.*, 1998; Wierdl *et al.*, 1997). For those presented in this study, the mutation rates are not known. Likewise for NPs, rate changes will depend on whether they are coding or non-coding, synonymous or non-synonymous, as well as other factors. In addition, both types of markers are influenced by physical linkage. For example, in the present study at least six of the NPs analysed are closely linked. Loci F16N1.1–1.6 are all located within a single 1800 bp fragment and only 12 genotypes are found in this region out of a possible 729 types. We believe that this would not be unexpected considering the physical proximity of the markers. Interestingly, there does not appear to be a positive correlation between physical distance and observed recombination in this region, as one might expect. Also of interest, in regard to the present findings, locus F16N1.1 is one of the two NP loci that subdivides group A. We believe, therefore, that additional approaches are needed to gain a better understanding of phylogenetic relatedness and to address such issues as the timing of clade divergence. Through this, we will better understand those factors influencing genetic diversity in this medically important species.

ACKNOWLEDGEMENTS

We would like to thank an anonymous reviewer for critical reading of the manuscript. This work was supported in part through a postdoctoral training fellowship awarded through the National Research Council of Egypt to M. M. E.

REFERENCES

- Bretagne, S., Costa, J. M., Besmond, C., Carsique, R. & Calderone, R. (1997). Microsatellite polymorphism in the promoter sequence of the elongation factor 3 gene of *Candida albicans* as the basis for a typing system. *J Clin Microbiol* **35**, 1777–1780.

- Chu, W. S., Magee, B. B. & Magee, P. T. (1993). Construction of an *Sfi*I macrorestriction map of the *Candida albicans* genome. *J Bacteriol* **175**, 6637–6651.
- Felsenstein, J. (1992). Phylogenies from restriction sites, a maximum likelihood approach. *Evolution* **46**, 159–173.
- Field, D., Eggert, L., Metzgar, D., Rose, R. & Wills, C. (1996). Use of polymorphic short and clustered coding-region microsatellites to distinguish strains of *Candida albicans*. *FEMS Immunol Med Microbiol* **15**, 73–79.
- Forche, A., Schonian, G., Graser, Y., Vilgalys, R. & Mitchell, T. G. (1999). Genetic structure of typical and atypical populations of *Candida albicans* from Africa. *Fungal Genet Biol* **28**, 107–125.
- Gerberding, J., Horan, T., Abshire, J. & 13 other authors (1999). National nosocomial infections surveillance (NNIS) system report, data summary from January 1990–May 1999, issued June 1999. *Am J Infect Control* **27**, 520–532.
- Graser, Y., Volosek, M., Arrington, J., Schonian, G., Presber, W., Mitchell, T. G. & Vilgalys, R. (1996). Molecular markers reveal that population structure of the human pathogen *Candida albicans* exhibits both clonality and recombination. *Proc Natl Acad Sci USA* **93**, 12473–12477.
- Hartl, D. L. & Clark, A. G. (1997). *Principles of Population Genetics*, 3rd edn. Sunderland, MA: Sinauer Associates.
- Hoyer, L. L., Scherer, S. & Shatzman, A. R. (1995). *Candida albicans* ALS1: domains related to a *Saccharomyces cerevisiae* sexual agglutinin separated by a repeating motif. *Mol Microbiol* **15**, 39–54.
- Hull, C. M., Raisner, R. M. & Johnson, A. D. (2000). Evidence for mating of the 'asexual' yeast *Candida albicans* in a mammalian host. *Science* **289**, 307–310.
- Johnson, E. M., Warnock, D. W., Luker, J., Porter, S. R. & Scully, C. (1995). Emergence of azole drug resistance in *Candida* species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidosis. *J Antimicrob Chemother* **35**, 103–114.
- Kao, A. S., Brandt, M. E., Pruitt, W. R. & 9 other authors (1999). The epidemiology of candidemia in two United States cities: results of a population-based active surveillance. *Clin Infect Dis* **29**, 1164–1170.
- Klein, R. S., Harris, C. A., Burkus, C., Small, B., Moll, B., Lesser, M. & Frieland, G. H. (1984). Oral candidiasis in high risk patients as the initial manifestation of the acquired immunodeficiency syndrome. *N Engl J Med* **311**, 354–358.
- Lott, T. J., Logan, D., Holloway, B., Fundyga, R. & Arnold, J. (1999). Towards understanding the evolution of the human commensal yeast, *Candida albicans*. *Microbiology* **145**, 1137–1143.
- McCullough, M. J., Clemons, K. V. & Stevens, D. A. (1995). Molecular epidemiology of the global and temporal diversity of *Candida albicans*. *Clin Infect Dis* **29**, 1220–1225.
- Magee, B. B. & Magee, P. T. (2000). Induction of mating in *Candida albicans* by construction of MTL α and MTL α strains. *Science* **289**, 310–313.
- Mercure, S., Montplaisir, S. & Lemay, G. (1993). Correlation between the presence of a self-splicing intron in the 25S rDNA of *C. albicans* and strains susceptibility to 5-fluorocytosine. *Nucleic Acids Res* **21**, 6020–6027.
- Miret, J. J., Pessoa-Brandao, L. & Lahue, R. S. (1998). Orientation-dependent and sequence-specific expansions of CTG/CAG trinucleotide repeats in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **95**, 12438–12443.
- Pfaller, M. A. (1995). Epidemiology of candidiasis. *J Hosp Infect Suppl* **30**, 329–338.
- Pujol, C., Reynes, J., Renaud, F., Raymond, M., Tibayrenc, M., Ayala, F., Janbon, F., Mallie, M. & Bastide, J.-M. (1993). The yeast *Candida albicans* has a clonal mode of reproduction in a population of infected human immunodeficiency virus-positive patients. *Proc Natl Acad Sci USA* **90**, 9456–9459.
- Pujol, C., Joly, S., Lockhart, S. R., Noel, S., Tibayrenc, M. & Soll, D. R. (1997). Parity among the randomly amplified polymorphic DNA method, multilocus enzyme electrophoresis, and Southern blot hybridization with the moderately repetitive DNA probe Ca3 for fingerprinting *Candida albicans*. *J Clin Microbiol* **35**, 2348–2358.
- Rustchenko-Bulgac, E. P. (1991). Variations of *Candida albicans* electrophoretic karyotypes. *J Bacteriol* **173**, 6586–6596.
- Schmid, J., Herd, S., Hunter, P. R. & 14 other authors (1999). Evidence for a general-purpose genotype in *Candida albicans*, highly prevalent in multiple geographical regions, patient types and types of infection. *Microbiology* **145**, 2405–2413.
- Schneider, S., Kueffer, J.-M., Roessli, D. & Excoffier, L. (1997). Arlequin version 1.1: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Swofford, D. L. (1998). PAUP*. Phylogenetic analysis using parsimony (*and other methods). version 4. Sunderland, MA: Sinauer Associates.
- Thrash-Bingham, C. & Gorman, J. A. (1992). DNA translocations contribute to chromosome length polymorphisms in *Candida albicans*. *Curr Genet* **22**, 93–100.
- Tibayrenc, M. (1997). Are *Candida albicans* natural populations subdivided? *Trends Microbiol* **5**, 253–254.
- Vilgalys, R., Graser, Y. & Mitchell, T. G. (1997). Response from Vilgalys et al. *Trends Microbiol* **5**, 254–257.
- Wierdl, M., Dominska, M. & Petes, T. D. (1997). Microsatellite instability in yeasts: dependence on the length of the microsatellite. *Genetics* **146**, 769–779.
- Xu, J., Boyd, C. M., Livingston, E., Meyer, W., Madden, J. F. & Mitchell, T. (1999a). Species and genotypic diversities and similarities of pathogenic yeasts colonizing women. *J Clin Microbiol* **37**, 3835–3843.
- Xu, J., Mitchell, T. G. & Vilgalys, R. (1999b). PCR-restriction fragment length polymorphisms (RFLP) analyses reveal both extensive clonality and local genetic differences in *Candida albicans*. *Mol Ecol* **8**, 59–73.
- Yang, R.-C. & Yeh, F. C. (1993). Multilocus structure in *Pinus contorta* Dougl. *Theor Appl Genet* **87**, 568–576.

Received 4 August 2000; revised 28 February 2001; accepted 9 March 2001.