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Diversity of DNA Fingerprints in *Cryptococcus neoformans*

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DNA fingerprint patterns of 156 *Cryptococcus neoformans* isolates (26 AIDS patients, 46 non-AIDS patients, and 40 environmental sources) from both varieties (126 *C. neoformans* var. *neoformans* and 30 *C. neoformans* var. *gattii* isolates) and from seven countries were analyzed by using the DNA probe UT-4p. Nine and twelve distinct DNA fingerprint patterns were observed for isolates of the *C. neoformans* var. *neoformans* and var. *gattii*, respectively. No pattern was unique to AIDS patients, non-AIDS patients, or the environment. Pattern II was observed more often in non-AIDS patients (8 of 23) than in AIDS patients (0 of 25). Pattern V was the most prevalent pattern (42 of 82) in clinical and environmental isolates. Isolates from three AIDS patients in Burundi and Zaire exhibited patterns identical to each other but different from those of isolates collected from their houses (i.e., dust of floors, walls, etc.) or a nearby pigeon coop. DNA fingerprint stability was determined for 53 isolates from nine non-AIDS patients at different time intervals during 5 to 128 weeks of antifungal therapy. For eight patients, the fingerprint pattern was stable while the ninth may have had a mixed infection. Pattern II was observed in 4 of 9 patients, which is similar to 4 of 14 in other non-AIDS patients as reported here. In spite of the extensive pattern heterogeneity among 15 *C. neoformans* var. *gattii* isolates in Australia, the patterns observed in seven California isolates were quite different from those in Australia. Among isolates of *C. neoformans* var. *gattii*, one fingerprint pattern (designated b) was observed in several countries of the Far East. The fingerprint patterns of two of three environmental isolates from *Eucalyptus camaldulensis* trees in Australia were identical to those of 2 of the 12 clinical isolates from that country.

It has been found that AIDS patients infected with isolates of the *Cryptococcus neoformans* var. *neoformans* are predominantly of serotype A (10); however, serotype D isolates have also been reported in AIDS patients from France (6), Germany (20), and the United States (3). Previous studies using restriction fragment length polymorphisms of mitochondrial DNAs had revealed that the population of serotype A isolates causing infection in AIDS patients was no different from those causing infection in non-AIDS patients (10). In order to discriminate between individual isolates in a population which is morphologically and physiologically indistinguishable, a stable and sensitive genetic marker is needed. Such a marker would enable the identification of a clinical isolate's true environmental source, identify single-source outbreaks of cryptococcosis, and allow discrimination between a relapse and a reinfection.

Several typing approaches have been used in epidemiological studies, including serotyping (8, 9, 17, 27), electrophoretic karyotyping (13, 14), mitochondrial DNA probes (24), genomic DNA probes (6, 15, 18, 19, 26), allelic variations at the *URA5* locus (2), multilocus enzyme typing (1), creatinine utilization (20), and PCR fingerprinting (12). Serotyping schemes have been useful for the ecological and epidemiological differences between the two varieties, *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii*. The existence of chromosomal length polymorphisms has led to the use of electrophoretic karyotyping (13, 14) for comparisons of different isolates. Polymorphisms at the *URA5* locus (2, 4) and in mitochondrial DNA (24) have exposed the diversity that exists among *C. neoformans* isolates. DNA fingerprinting has demonstrated the ability to discriminate between closely related isolates within a given population (5, 26).

We previously reported (6, 26) the use of a genomic probe (UT-4p) for strain identification in *C. neoformans*. The DNA fingerprint patterns were observed to be stable and provided the degree of resolution necessary to determine genetic diversity and relatedness among closely related isolates of *C. neoformans*. In the present study, the probe UT-4p has been used to examine the diversity of DNA patterns in both varieties (*C. neoformans* var. *neoformans* and var. *gattii*) of this organism in clinical and environmental isolates recovered from different parts of the world. Stability of the DNA fingerprint patterns was also determined for isolates recovered from patients undergoing prolonged antifungal therapy.

MATERIALS AND METHODS

Strains and media. The sources and geographical locations of the *C. neoformans* strains used in this study are listed in Table 1. These strains had been cultured from either AIDS patients (25 with *C. neoformans* var. *neoformans*; 1 with *C. neoformans* var. *gattii*), non-AIDS patients (23 with *C. neoformans* var. *neoformans*; 23 with *C. neoformans* var. *gattii*), or environmental sources (34 of *C. neoformans* var. *neoformans*; 6 of *C. neoformans* var. *gattii*).

Of the 33 *C. neoformans* var. *neoformans* isolates obtained from southern California, 14 were from AIDS patients, 14 were from non-AIDS patients, and 5 were from environmental sources. The five environmental isolates had been recovered from *Eucalyptus camaldulensis* trees at three different sites: two in the San Diego zoo, one in Carlsbad (20 miles from the zoo), and two in Griffith Park (120 miles from the zoo and 100 miles from Carlsbad).

Of the German serotype A isolates used in this study, eight were obtained from AIDS patients in Berlin and 12 were environmental isolates obtained from droppings of a variety of birds collected at different locations in the city of Berlin which included the aviary of the zoo, private households, pigeon breeders, and pet shops (20). DNA fingerprints from three of eight isolates from AIDS patients were each compared with bird dropping isolates from specific areas to which the patients might have been exposed. These included clinical isolate 624, compared with isolates from droppings of budgerigar and parrots (isolates 641, 642, and 643) in a pet shop; clinical isolate 630, compared with a bird dropping isolate of a parrot (isolate 644) in a pet shop; and clinical isolate 626, compared with isolates from feral pigeon droppings (isolate 636) from the patient's neighborhood.

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TABLE 1. The sources of the 156 AIDS and non-AIDS isolates of *C. neoformans* used

C. neoformans var.	No. of isolates	Location	C/E[a]	Source
C. neoformans var. *neoformans*	33	California	28/5	D. H. Howard
	20	Berlin	8/12	F. Staib
	17	Burundi	2/15	D. Swinne
	3	Zaire	1/2	D. Swinne
	53[b]	NIH	53/0	(J. E. Bennett) NIH Clinical Center
C. neoformans var. *gattii*	7	California	4/3	D. H. Howard; D. Ellis
	15	Australia	12/3	R. McAleer; D. Ellis
	5	India	5/0	A. Padhye
	1	Japan	1/0	S. Kohno
	1	China	1/0	CBS[c]
	1	Africa	1/0	D. Swinne

[a] Ratio of clinical to environmental isolates.
[b] From nine different patients.
[c] CBS, Centraalbureau voor Schimmelcultures.

Twenty African isolates (17 from Bujumbura, Burundi, and 3 from Kinshasa, Zaire) from AIDS patients and their immediate surroundings were studied (Table 2).

Eleven clinical isolates of *C. neoformans* var. *gattii* from non-AIDS patients in Australia were provided by R. McAleer, and one isolate from an AIDS patient was provided by D. Ellis. Four isolates from non-AIDS patients in California were provided by D. Howard. All environmental isolates of *C. neoformans* var. *gattii* were obtained from *Eucalyptus camaldulensis* trees, three in California (one from San Francisco, two from San Diego) and three from the Barosa Valley in Australia (11). Eight of the remaining cultures, five from India and one each from Japan, China, and Africa, had been cultured from non-AIDS patients.

Fifty-three *C. neoformans* isolates from nine non-AIDS patients were obtained at intervals during 5 to 128 weeks of antifungal therapy at the National Institutes of Health (NIH). These isolates had been stored lyophilized soon after isolation.

All other strains were maintained on YEPD (1% yeast extract, 2% Bacto Peptone, 2% glucose) agar slants at 25°C. Varietal status of the isolates was determined by CGB medium (9). The serotypes of all the isolates collected were confirmed by one of the authors by the whole-cell agglutination procedure (27).

DNA probes. The linear DNA plasmid UT-4p was extracted from the *URA5* transformant of B-4476-FOA as has been described earlier (23, 26).

Southern analysis. Genomic DNA was extracted (25) from each of the selected isolates, and 3 to 5 μg of the DNA was electrophoresed on 0.8% agarose after digestion with the restriction endonuclease *Acc*I. The DNA was then transferred to Nytran filters (Schleicher & Schuell, Keene, N.H.) by the method of Southern (16). Radiolabelled probes of UT-4p were prepared by using a random-priming kit (Stratagene, La Jolla, Calif.) and [^{32}P]dCTP (Amersham, Oak Park, Ill.). The filters were then hybridized, washed, and exposed to X-ray film as described earlier (23, 26).

Pattern similarities and differences. Restriction fragment length polymorphism is a developing science, and precise differences and their meaning are as yet uncertain. DNA fingerprint patterns generated by Southern hybridizations often exhibit variations introduced by technical aspects of the procedures used. Artifacts, smiles, shifts, incomplete digests, or nonspecific hybridizations are eliminated by reproducing the patterns several times before analyzing the data. The presence or absence of a major (dark) band was considered a difference. Generally, all minor (light) bands, except those that are really faint, were considered in the analysis. As an exception, the presence of one minor band in A23 was ignored since the overall pattern of major bands was identical in that strain.

RESULTS

DNA fingerprint patterns were analyzed for 156 *C. neoformans* isolates from different geographical areas. Of these isolates, 126 were *C. neoformans* var. *neoformans* while 30 were *C. neoformans* var. *gattii* (Table 1). With only one exception, all the strains isolated from AIDS patients were determined to be

TABLE 2. *C. neoformans* isolates collected in Bujumbura, Burundi (22), and Kinshasa, Zaire (20)

Location and culture no.	C/E[a]	DNA pattern	Source
Bujumbura, Burundi			
B-4483	C	VI	AIDS patient 2
B-4485	E	II	Patient 2's bedroom furnishings
B-4517	E	V	Patient 2's kitchen floor
B-4518	E	II	Patient 2's living room (under roof)
B-4519	E	II	Patient 2's living room floor
B-4520	E	V	Patient 2's bedroom dressing table
B-4484	C	VI	AIDS patient 3
B-4487	E	I	Dust from walls of patient 3's house
B-4522	E	I	Pigeon droppings from nearby coop
B-4521	E	V	Dust from a control[b] house
B-4510	E	V	Dust from a control house
B-4512	E	V	Dust from a control house
B-4513	E	V	Dust from a control house
B-4514	E	V	Pigeon droppings in a coop
B-4515	E	V	Dust from house A near the coop
B-4516	E	V	Dust from house A near the coop
B-4525	E	V	Dust from house B near the coop
Kinshasa, Zaire			
B-4482	C	VI	Patient 4
B-4486	E	II	Patient 4's house dust
B-4523	E	V	Patient 4's house dust

[a] Type of isolate: clinical (C) or environmental (E).
[b] House in which no infected person resides.

TABLE 3. The geographic sources of different patterns from AIDS, non-AIDS, and environmental isolates of *C. neoformans* var. *neoformans* used

Pattern	Total no. of isolates with pattern	No. of isolates[a] with pattern from:									
		California			Berlin		Burundi		Zaire		NIH[b]
		A	NA	E	A	E	A	E	A	E	
I	6	1	1					2			1
II	15		4		2	1		3		1	4
III	5	3	2								
IV	7	3	2								2
V	42	5	4	5	5	11		10		1	1
VI	3						2		1		
VII	1		1								
VIII	2	1			1						
IX	2	1									1
Total	83	14	14	5	8	12	2	15	1	2	9[c]

[a] A, isolates from AIDS patients; NA, isolates from non-AIDS patients; E, environmental isolates.
[b] Isolates from nine non-AIDS patients at NIH.
[c] Two patterns were observed for one patient.

serotype A (*C. neoformans* var. *neoformans*). The primary environmental source of *C. neoformans* var. *neoformans* isolates in southern California was pigeon droppings. Of the 30 *C. neoformans* var. *gattii* isolates analyzed, 23 were cultured from non-AIDS patients from various geographic regions while only one of the isolates had been recovered from an AIDS patient in Melbourne, Australia. The remaining six were environmental isolates which had been recovered from *Eucalyptus camaldulensis* trees, three in Australia and three in southern California.

***C. neoformans* var. *neoformans*.** With a DNA probe described previously (26), nine distinct DNA fingerprint patterns (designated I to IX) were observed among all the clinical and environmental isolates of *C. neoformans* var. *neoformans* (Table 3; Fig. 1). Patterns V, VI, and VII were similar to each other. Isolates exhibiting pattern V appeared to be the most prevalent (27 of 34) type of environmental isolates in California, Burundi, Zaire, and Berlin (Table 3). This DNA pattern also appeared to be well represented in isolates from AIDS and non-AIDS patients in California (Fig. 2) and AIDS patients in Berlin. The DNA fingerprint patterns among isolates from California were very heterogeneous in both AIDS and non-AIDS populations. The distribution of four (I, III, IV, V) patterns in clinical isolates from southern California was independent of underlying AIDS. Of the nine fingerprint patterns, the only patterns not observed among the non-AIDS isolates in California were VI, VIII, and IX while those not observed among AIDS isolates were II, VI, and VII. Isolates from AIDS patients displaying fingerprint patterns VII and IX were seen only in California. It may be that the number of isolates obtained from Burundi and Zaire was too small to include these patterns. Pattern VI was observed only among the three isolates from AIDS patients obtained in the two African countries. It is significant to note that the three isolates had identical patterns and that this pattern was not found in the isolates which had been recovered from the dust (floor, walls, furniture, etc.) in and around the patients' homes (Fig. 3A) or from a nearby coop (Fig. 3B).

Of the three AIDS patients from different parts of Berlin, two (isolates 624 and 630) were believed to be frequently

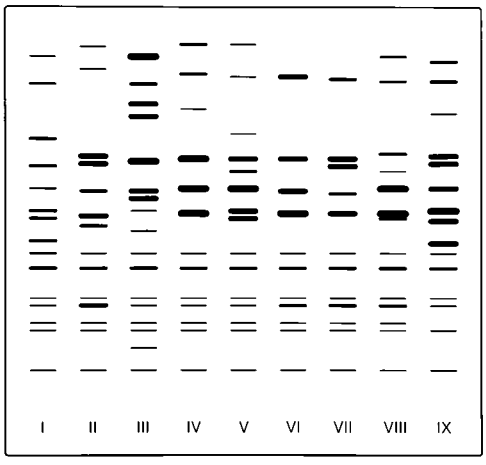

FIG. 1. Graphic representation of the nine distinct DNA fingerprint patterns obtained from clinical and environmental isolates of *C. neoformans* var. *neoformans* serotype A.

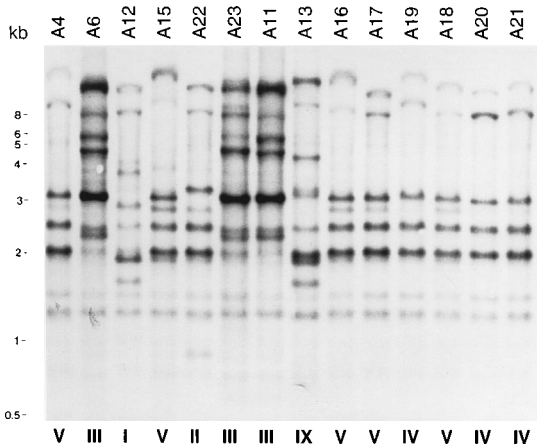

FIG. 2. DNA fingerprint patterns of *C. neoformans* var. *neoformans* isolates from non-AIDS patients in California by Southern blot hybridizations. Genomic DNAs from each isolate were digested with *Acc*I and hybridized to a radiolabelled probe of the plasmid UT-4p. Labels at the bottom of each lane correspond to one of the patterns shown in Fig. 1. DNA size markers on the left represent kilobases of DNA and are derived from the 1-kb lambda DNA ladder.

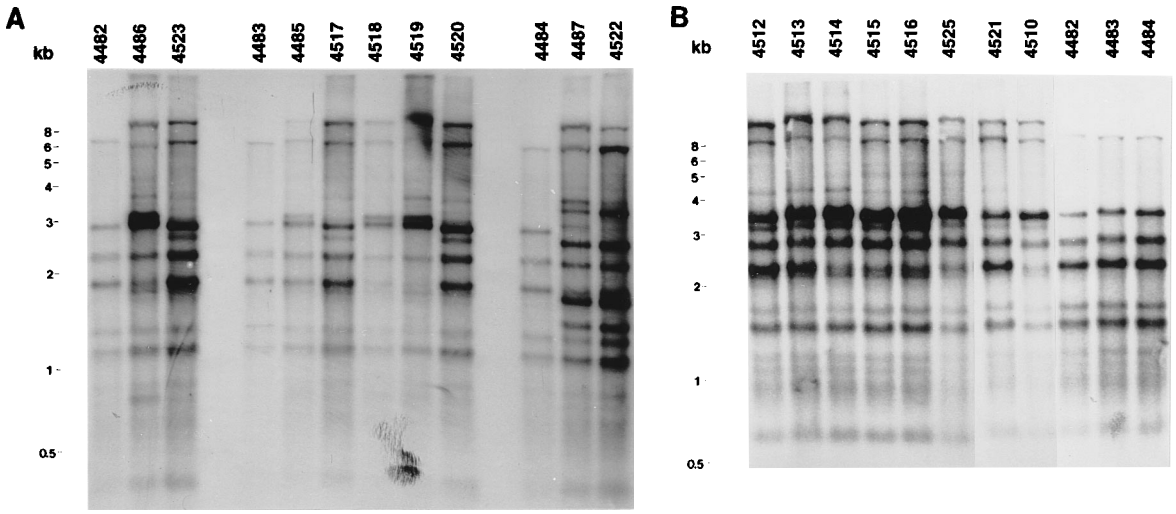

FIG. 3. DNA fingerprint patterns of *C. neoformans* var. *neoformans* strains in Zaire obtained from AIDS isolates B-4482, B-4483, and B-4484 and a variety of dust samples from the patients' immediate surroundings (Table 2) (A) and environmental isolates from a nearby pigeon coop (B). Genomic DNAs from each isolate were digested with *Acc*I and hybridized to a radiolabelled probe of the plasmid UT-4p. DNA size markers on the left represent kilobases of DNA and are derived from the 1-kb lambda DNA ladder.

exposed to bird droppings of caged birds (budgerigar and parrot, isolates 641 and 643, respectively, and parrot, isolate 644) while one (isolate 626) was often exposed to droppings of feral pigeons (638). The DNA fingerprints of the two patients exposed to droppings of caged birds were observed to be identical (Fig. 4). In the case of the patients believed to have been exposed to droppings of feral pigeons, however, the DNA fingerprint patterns were different (Fig. 4).

Including the isolates from NIH, isolates exhibiting pattern II were found significantly more often in non-AIDS (8 of 23) than in AIDS (0 of 25) patients. Pattern V isolates were recovered more often from the environment (27 of 34) than from clinical sources (15 of 48). The latter could also be an artifact generated from collections of multiple isolates from a single site, as may be the case for the pigeon coop isolates in Burundi. Isolates obtained from excreta of different birds taken at the aviary in the Berlin zoo also showed pattern V to be the most prevalent type. In California, pattern V was the only type recovered from the environment.

***C. neoformans* var. *gattii*.** Twelve discernible fingerprint patterns (designated a to l) were identified among the 30 clinical and environmental isolates of *C. neoformans* var. *gattii* (Fig. 5). Almost half of our sample (17 of 30), which was largely from overseas, was determined to contain seven different DNA fin-

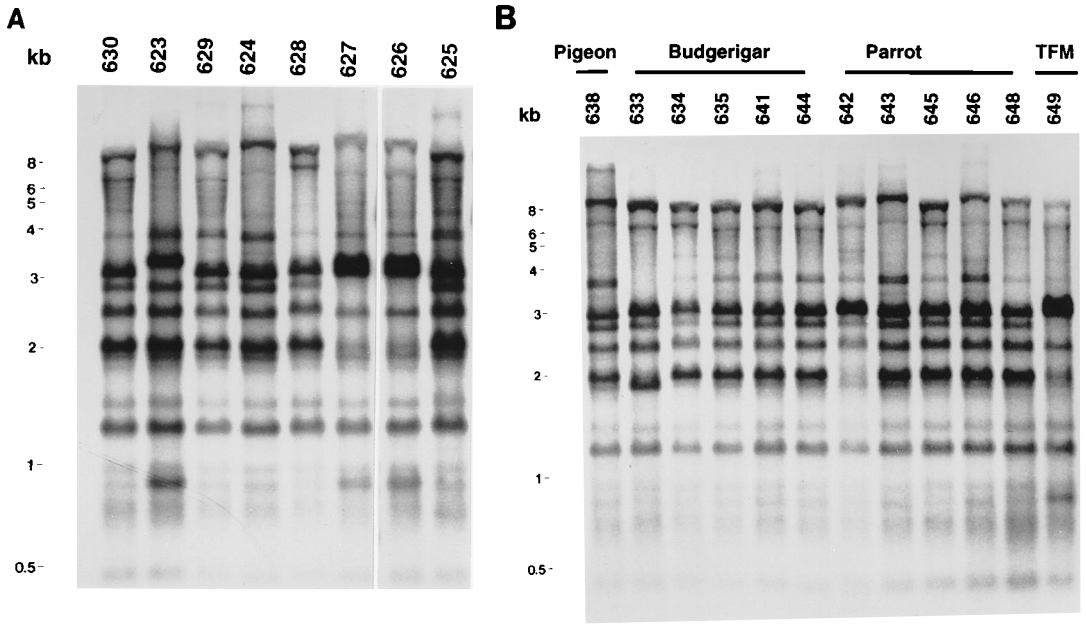

FIG. 4. DNA fingerprint patterns of *C. neoformans* var. *neoformans* strains in Berlin obtained from AIDS patients (A) and environmental isolates from different locations in Berlin (B). Genomic DNAs from each isolate were digested with *Acc*I and hybridized to a radiolabelled probe of the plasmid UT-4p. DNA size markers on the left represent kilobases of DNA and are derived from the 1-kb lambda DNA ladder. TFM, tawny frogmouth (nocturnal bird).

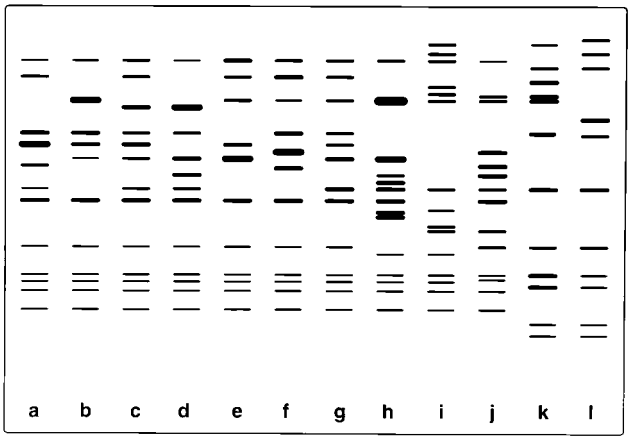

FIG. 5. Graphic representation of the 12 (a to l) distinct DNA fingerprint patterns obtained from clinical and environmental isolates of *C. neoformans* var. *gattii* serotype B.

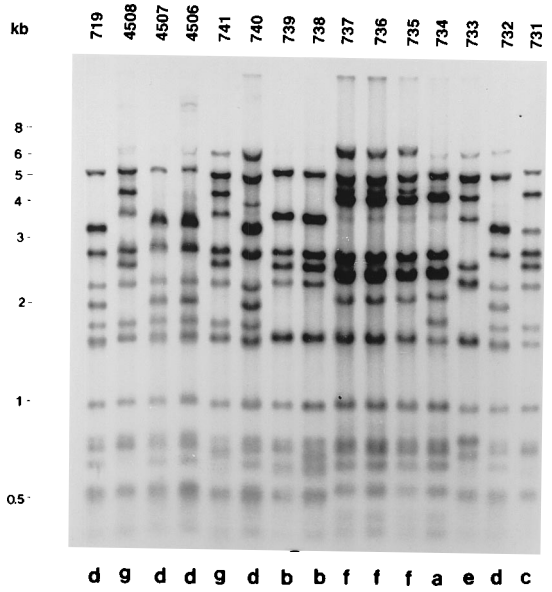

FIG. 6. DNA fingerprint patterns of *C. neoformans* var. *gattii* strains obtained from an AIDS patient (719), non-AIDS patients (731 to 741), and *Eucalyptus* tree isolates (4506, 4507, and 4508) in Australia. Genomic DNAs from each isolate were digested with *Acc*I and hybridized to a radiolabelled probe of the plasmid UT-4p. Labels at the bottom of each lane correspond to one of the patterns shown in Fig. 4. DNA size markers on the left represent kilobases of DNA and are derived from the 1-kb lambda DNA ladder.

gerprint patterns (a to g) (Table 4). The DNA fingerprint of two pattern d isolates, which had been recovered from the debris of *Eucalyptus* trees, matched the fingerprints of three clinical isolates (Fig. 6). The significance of this observation is unknown since no exposure history of these patients is available. The DNA fingerprint of another environmental isolate (pattern g) was found to match that of a clinical isolate from a non-AIDS patient (Fig. 6, lanes 4508 and 741), although no exposure history is available to link the two isolates. Isolates of pattern b, which were the most prevalent (7 of 30), were also consistently observed in other countries of the Far East (Table 4).

The fingerprint patterns of the seven Californian isolates were different from those exhibited by the Australian isolates although the number of isolates from California was too small to attach much significance to this observation. Three patterns (h, i, and k) were observed among the seven isolates recovered from California. Of these, pattern i was found to be most prevalent (five of seven). Two of the three environmental isolates from *Eucalyptus* trees in California were of pattern i and matched two clinical isolates from non-AIDS patients from that state.

DNA fingerprints from patients undergoing different drug therapies. DNA fingerprints of 53 clinical isolates of *C. neoformans* var. *neoformans* from nine non-AIDS patients were analyzed (Table 5). In one patient, the fingerprint patterns of isolates cultured three weeks after initiation of therapy and 22 days after completion of 20 weeks of therapy gave identical patterns. The pretreatment and midtherapy isolates differed from the other two but resembled each other. The DNA fingerprints of isolates cultured during the first and the third visits were similar to each other (Fig. 7). This suggests the possibility of infection by more than one cryptococcal strain. All the other patients had consistent DNA fingerprint patterns (Table 5).

TABLE 4. The geographic sources of the different patterns among AIDS, non-AIDS, and environmental isolates of *C. neoformans* var. *gattii* (serotype B) used

Pattern	Total no. of isolates with pattern	No. of isolates[a] with pattern from:								
		Australia			California		India, NA	Japan, NA	China, NA	Africa, NA
		A	NA	E	NA	E				
a	1		1							
b	7		2				3	1	1	
c	1		1							
d	5	1	2	2						
e	1		1							
f	3		3							
g	2		1	1						
h	1					1				
i	5				3	2				
j	2						2			
k	1				1					
l	1									1
Total	30	1	11	3	4	3	5	1	1	1

[a] A, isolate from an AIDS patient; NA, isolates from non-AIDS patients; E, environmental isolate.

TABLE 5. Fifty-three isolates of serotype A *C. neoformans* obtained during prolonged therapy from nine non-AIDS patients

Drug and patient	Isolate no.	Week	Source	Pattern
Amphotericin B				
3	145	0	CSF[a]	II
	154	1	CSF	II
	147	2	CSF	II
	148	3	CSF	II
	153	4	CSF	II
	155	5	CSF	II
	162	6	CSF	II
	159	7	CSF	II
	163	8	CSF	II
	164	13	CSF	II
	165	13	CSF	II
	166	15	CSF	II
	175	23	CSF	II
5	065	0	CSF	II
	067	31	CSF	II
1	227	0	CSF	IX
	228	3	CSF	I
	262	8	CSF	IX
	260	20	CSF	I
6	068	0	CSF	II
	069	7	CSF	II
	269	107	CSF	II
Amphotericin B plus flucytosine				
7	140	0	CSF	I
	144	7	CSF	I
	146	8	CSF	I
	149	9	CSF	I
	152	10	CSF	I
	152	11	CSF	I
	156	12	CSF	I
	158	13	CSF	I
	160	16	Sputum	I
	261	86	Urine	I
	291	128	Urine	I
	292	128	Spleen	I
8	167	0	CSF	V
	168	6	CSF	V
	266	56	CSF	V
	284	94	CSF	V
9	203	0	CSF	II
	204	35	CSF	II
	223	36	CSF	II
	224	36	CSF	II
	280	90	CSF	II
Flucytosine				
10	490	0	Skin lesion	IV
	512	2	Skin lesion	IV
	513	3	Skin lesion	IV
	514	4	Skin lesion	IV
	515	5	Skin lesion	IV
11	484	0	CSF	IV
	480	2	CSF	IV
	481	2	CSF	IV
	482	3	CSF	IV
	483	5	CSF	IV

[a] CSF, cerebrospinal fluid.

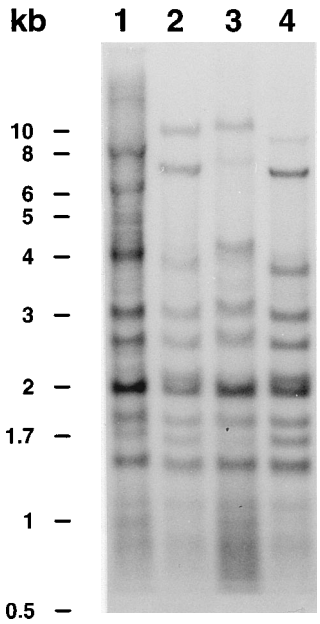

FIG. 7. DNA fingerprint patterns of *C. neoformans* var. *neoformans* strains obtained at different time intervals (weeks) from the non-AIDS patient 1 (Table 5) undergoing therapy at NIH. Lane 1, pretreatment; lane 2, 3 weeks; lane 3, 8 weeks; lane 4, 20 weeks. Genomic DNAs from each isolate were digested with *Acc*I and hybridized to a radiolabelled probe of the plasmid UT-4p. DNA size markers on the left represent kilobases of DNA and are derived from the 1-kb lambda DNA ladder.

DISCUSSION

By using the DNA probe UT-4p, which was developed and characterized earlier (26), we attempted to answer some fundamental questions about genetic diversity in *C. neoformans*. Is there a correlation between clinical isolates and those present in the immediate environment of patients? Within varieties, are there marked geographic differences between isolates? Are AIDS patients predisposed to infection by specific strains? The answer to these questions appears to be no.

In accordance with earlier reports, all but one isolate from AIDS patients were *C. neoformans* var. *neoformans*. Among patients infected with *C. neoformans* var. *neoformans*, pattern II was found more often in non-AIDS than in AIDS patients (8 of 23 versus 0 of 25) (Fisher's exact test: $P < 0.003$).

Of the nine different fingerprint patterns in *C. neoformans* var. *neoformans* isolates, pattern V appeared to be the most prevalent (42 of 82) in clinical as well as environmental isolates (from pigeon droppings and the debris of *Eucalyptus* trees). The isolation of a *C. neoformans* var. *neoformans* strain from the debris of *Eucalyptus camaldulensis* is not surprising since the debris could have been contaminated with avian guano containing the pattern V strain. The predominance of pattern V could be an artifact induced by the collection of many isolates from a few environmental sites such as pigeon coops. However, the isolates at the Berlin zoo were recovered at different times and from cages of different birds which were physically separated from each other, albeit all within the same aviary (20). The Californian isolates were recovered from three different and distant locations in southern California.

It was interesting to note that three clinical isolates from AIDS patients in Africa (two from Burundi and one from Zaire) exhibited DNA fingerprints identical to each other but different from those of isolates recovered from the dust in and around their houses or from a nearby pigeon coop. This may suggest a different and as yet unknown source of infection for these patients. Environmental isolates from the same environmental sites in Burundi and Berlin do exhibit some restricted heterogeneity in their fingerprint patterns. This suggests that the fungus may be spread by birds (20) or the wind between nearby sites (21).

For the two AIDS patients from Berlin who were presumably exposed to caged birds (budgerigar and parrots), the patterns of their DNA fingerprints were identical. The creatinine auxanogram of these isolates has also been reported to be identical (20). It is possible that these two patients were infected from avian sources such as those in the environmental samples. The DNA fingerprints in this study did not allow any distinction between isolates from droppings of caged or free-flying birds since pattern V was the predominant pattern in clinical as well as environmental isolates regardless of their creatinine auxanogram patterns.

Isolates of *C. neoformans* var. *gattii* have primarily been isolated either from the debris of *Eucalyptus* trees in the environment or from non-AIDS patients. The only isolate of *C. neoformans* var. *gattii* that was isolated from an AIDS patient was in Melbourne, Australia, and was typed as serotype B. Most environmental *C. neoformans* var. *gattii* isolates have been recovered from the debris of *Eucalyptus* trees in Australia, with a small number obtained from California. All have been serotype B (7, 11). There is more heterogeneity in this variety (12 distinct patterns in just 30 isolates) than in *C. neoformans* var. *neoformans*. The mechanism of spread of *C. neoformans* var. *gattii* in the environment is as yet unknown. It could be useful to learn whether pattern similarities are limited to isolates from the same tree, neighboring trees, or trees in adjacent groves or extend to trees of different geographic areas. Furthermore, no such correlation has been demonstrated between regional clinical and environmental isolates. While the patterns of environmental isolates in California were different from those in Australia, the patterns were observed in some clinical isolates from their respective areas. Pattern d, observed in the only *C. neoformans* var. *gattii* isolate from an Australian AIDS patient, was also observed in two of three environmental isolates. Similarly, pattern i was often observed in clinical (three of four) as well as environmental (two of three) isolates. As in *C. neoformans* var. *neoformans*, fingerprint patterns in isolates of *C. neoformans* var. *gattii* were also observed in the environmental isolates.

The geographic distribution of DNA patterns among serotype D patient isolates has recently been reported in France (5). As is the case in the present study, no significant clustering was found. Predominance of our clinical isolates exhibiting either of the fingerprint patterns II and V, however, was notable.

The stability of an isolate's fingerprint pattern while in the host is critical in allowing subsequent discrimination between a recurrence and a new infection. We and others have reported previously (4, 24) that populations of *C. neoformans* isolated from each patient were homogeneous. Stability of the DNA fingerprints was analyzed in isolates from patients undergoing a variety of drug treatments. Isolates were recovered at different time intervals, from several patients, before and during treatment with amphotericin B, flucytosine, or both. For eight of nine patients, the DNA fingerprints remained unaltered for as long as 2 years. An apparent change in the DNA fingerprints of a strain was noted for one patient undergoing treatment. This change could be the result of either the mislabelling of cultures, a mixed initial infection by more than one isolate, or technical problems, such as incomplete digestion. Since the patterns were reproducible, the changes may represent the inadvertent sampling of a minority population. The latter would argue for the analysis of more than one isolate for confirmation.

The DNA fingerprints generated by the probe UT-4p appear to have sufficient discriminatory power to be used as a tool for epidemiological studies of *C. neoformans*. Although other techniques such as karyotyping and rapid amplification of polymorphic DNA may allow more sensitivity, UT-4p exhibits the ability to discriminate among isolates of all four serotypes. In spite of the relatively low sample sizes and the lack of sufficient knowledge about the host-environment interaction leading to infection, such studies do provide a useful approach to understanding the probable source and thereby the mode of infection with *C. neoformans.*

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