PCR fingerprinting of *Trichophyton mentagrophytes* var. *interdigitale* using polymorphic subrepeat loci in the rDNA nontranscribed spacer

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The sequence of the nontranscribed spacer (NTS) region of the rDNA of *Trichophyton mentagrophytes* var. *interdigitale* strain 2111 was determined, and three individual subrepeat loci identified. The first repeat region contained eight tandem copies of a degenerate 33–43 bp sequence, whilst the second had two complete and two partial 300 bp repeats. The third locus contained six tandemly repetitive elements of between 67 and 89 bp, which showed sequence identity to the TrS2 repeats of *Trichophyton rubrum*. PCR amplification of the individual repetitive regions from 42 random isolates of *T. mentagrophytes* var. *interdigitale* identified fragment length polymorphisms at each locus. Sequence analysis of the PCR products revealed that the size variations resulted from differences in the copy number of each of the three sets of subrepeat elements, TmiS0, TmiS1 and TmiS2. In addition, some indels were present in the flanking regions of the TmiS1 repeats. Combining PCR fingerprints from each of the three polymorphic loci produced a total of 19 individual strain profiles. The method was rapid, reproducible and discriminatory, and the fragment patterns simple to interpret. PCR fingerprint analysis of variable tandem repeat loci in the *T. mentagrophytes* var. *interdigitale* NTS represents a valuable molecular typing method for future epidemiological investigations in this species.

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INTRODUCTION

Human-adapted dermatophyte species are the most prevalent fungal pathogens in the developed world. Although the clinical consequences of dermatophyte infections are generally benign, and serious sequelae from these infections are rare, medical costs associated with the treatment of dermatophytoses place a large burden on healthcare budgets. These have been conservatively estimated at \$400 million per annum in the USA alone (Drake *et al.*, 1996; Smith *et al.*, 1998). High rates of dermatophyte infection are maintained by widespread use of communal recreational facilities, such as gymnasiums, sports centres and public swimming baths. These environments afford optimal conditions for the transmission of tinea infections,

particularly ‘athlete’s foot’ (tinea pedis). Once an infection is established, the wearing of sports shoes and other types of occlusive footwear provides an ideal microclimate in which these fungi can thrive, and may lead to nail infections (tinea unguium), which are inherently harder to treat. It has been estimated that there is a relapse rate of 10 % a year in cases of tinea pedis treated with oral terbinafine (Takiuchi *et al.*, 2005), while 22 % of patients with tinea unguium treated with systemic antifungals relapse within 3 years (Tosti *et al.*, 1998). The design of effective intervention measures to break the cycle of transmission is a central requirement for reducing the incidence of dermatophytoses. The epidemiological typing of fungal isolates is one means by which point sources of infection, routes of transmission, and sources of recurrence (i.e. reinfection with new strains or recrudescence of the original strain) can be accurately identified.

The two most common agents of tinea pedis are the anthropophilic dermatophytes *Trichophyton mentagrophytes* var. *interdigitale* and *Trichophyton rubrum* (Zaias & Rebell, 2003). Both species cause very similar clinical conditions, but are quite well separated in the phylogeny

Abbreviations: LSU, large subunit; NTS, nontranscribed spacer; SRE, subrepeat element; SSU, small subunit; VIR, variable internal repeat.

The GenBank/EMBL/DBJ accession number for the NTS region of *T. mentagrophytes* var. *interdigitale*, containing the coordinates of the three subrepeat loci, is DQ486866.

Supplementary data are available with the online version of this paper.

Table 1. Details of *T. mentagrophytes* var. *interdigitale* isolates used in this study

Abbreviations: KMU, Kanazawa Medical University; NK, not known; ND, not done; NR, no result.

Isolate	Origin	Site	Strain PCR type			RFLP* group
			S0	S1	S2	
Av.A27960†	Belgium	NK	B	0	I	ND
EK1471	UK	Chest	A	1	I	ND
EP0843	UK	NK	C	2	I	P-6
EP1127	UK	NK	I	7	IA	P-3
2882	UK	NK	G	1	II	P-1
0388	UK	NK	F	1	II	ND
1553	UK	NK	C	2	II	ND
T2.261	UK	NK	C	2	II	P-6
2111	UK	NK	C	2	II	P-1
Tmi.dysg	UK	NK	D	2	II	ND
0269	UK	NK	D	2	II	ND
T2.2450	NK	NK	D	2	II	ND
2768	UK	NK	G	2	II	ND
7435	UK	NK	D	2	II	ND
3964	UK	NK	D	2	II	ND
SM8796	Japan	Foot	D	2	II	P-1
KMU.Kdy	Japan	NK	D	2	II	P-1
KMU.Tnk	Japan	NK	D	2	II	P-1
KMU.Ysh	Japan	Foot	D	2	II	P-1
2710	UK	NK	D	2	II	P-1
EP1039	UK	Toe	D	2	II	P-1
EP1119	UK	Toe web	D	2	II	P-1
EP0904	UK	NK	D	2	II	ND
EP0939	UK	Foot	D	2	II	P-1
EP9294	UK	NK	D	2	II	ND
6679	UK	NK	D	2	II	P-1
KMU.Tkd	Japan	NK	F	2	II	P-1
EP0914	UK	Toenail	F	2	II	P-3
2035	UK	NK	D	3	II	P-9
KMU.Aik	Japan	Palm	D	4	II	P-5
4605	UK	NK	D	6	II	P-2
EK4354	UK	Chin	J	5	II	P-11
EP1159	UK	Nail	D	6	II	P-4
3083	UK	NK	D	6	II	P-4
T2.36	NK	NK	D	7	II	ND
4153	UK	NK	D	7	II	P-5
EG5704	UK	Shin	D	7	II	P12
EP1174	UK	Toenail	D	7	IV	P-2
EF2608	UK	Palm	E	8	III	P-10
3850	UK	NK	D	9	II	P-4
8070	UK	NK	H	10	II	P-7
SM8774‡	India	NK	NR	NR	II	P-1
SM8779‡	India	NK	NR	NR	II	P-1

*The RFLP type as determined using a probe targeted to the NTS region (Mochizuki *et al.*, 2003).

†Av.A27960, *A. vanbreuseghemii* isolate from Professor R. Vanbreuseghem, Antwerpen, Belgium.

‡The original designations for the two isolates from the Indian subcontinent were Menon 187 for SM8774 and Menon 302 for SM8779.

of the genus *T. mentagrophytes* var. *interdigitale* has been placed in a separate species, *T. interdigitale*, by some authors (Gräser *et al.*, 1999a), but this group of dermatophytes, isolated almost exclusively from the skin and nails of human feet, is distinctive regardless of name. Isolates of *T. mentagrophytes* var. *interdigitale* can show substantial variation in colony morphology and appearance, but these characters can alter greatly on subculture, and are therefore inappropriate for use as phenotypic strain markers.

Molecular subtyping offers an alternative way of identifying individual fungal isolates for epidemiological purposes. We have previously developed a genotyping procedure based upon variable tandem subrepeat elements (SREs) in the rDNA gene cluster of *T. rubrum* (Jackson *et al.*, 2000). Recently, *T. mentagrophytes* var. *interdigitale* has also been shown to possess genetic polymorphisms that map to the rDNA (Mochizuki *et al.*, 2003). Here, we report the molecular characterization of the nontranscribed spacer (NTS) of the rDNA of *T. mentagrophytes* var. *interdigitale*, and identify three variable loci within it that consist of tandemly repetitive subelements. PCR amplification of each of these polymorphic NTS loci generated simple, discriminatory patterns, which when combined produced a robust molecular subtyping scheme for this dermatophyte.

METHODS

Fungal isolates. The strains used and their origins are listed in Table 1. All were randomly selected isolates cultured from clinical samples submitted from patients with dermatophytoses, though the exact specimen type was not available in some cases. Twenty samples were obtained from the Mycology Reference Centre at Leeds University Medical School, Leeds, UK, and 14 from the Department of Dermatology at St Thomas's Hospital, London. Strains from Japan (six), India (two) and Belgium (one) were provided by the Department of Dermatology, Kanazawa Medical University, Japan. The clinical isolates were identified on the basis of microscopy and colony morphology from the primary cultures, and by restriction profiling of ITS regions 1 and 2 (Jackson *et al.*, 1999; Mochizuki *et al.*, 2003).

DNA isolation. Nucleic acids were extracted from fungal cultures as described previously (Jackson *et al.*, 1999), with the following modifications. The lysis buffer contained 100 µg proteinase K ml⁻¹, and the initial extraction of the lysate with ice-cold chloroform was omitted.

PCR amplification and cloning of the NTS region. The NTS region was successfully amplified from three *T. mentagrophytes* var. *interdigitale* isolates (2111, EP0914 and KMU.Aik) using a modification of the procedure described previously (Jackson *et al.*, 2000). The PCR reaction contained 1× Expand Long Buffer 3, 2.75 mM magnesium chloride, 500 µM each deoxynucleoside triphosphate (dATP, dCTP, dTTP, dGTP), 300 nM of each conserved primer [forward 25SCON2, reverse NS1-R (White *et al.*, 1990; Table 2)], 3.75 units of Expand Long high fidelity *Taq* DNA polymerase (Expand Long Template PCR System, Roche) and 1 µl genomic DNA template (~20 ng), in a total volume of 50 µl. The thermal profile was altered to optimize the yield, with an initial denaturation of 95 °C for 2.5 min, followed by ten cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 35 s and extension at 68 °C for 75 s.

Table 2. PCR primers used for amplification of the NTS region, and for the three fingerprinting assays.

Primer	Sequence
Primers for amplification of the Tmi NTS region	
Forward primer 25SCON2	5' TAG ACC GTC GTG AGA CAG
Reverse primer NS1-R	5' GAG ACA AGC ATA TGA CTA C
Primers for amplification of the S0 tandem repeat region	
Forward primer TmiS0F	5' CGA AGG ATA CTG TGG AAG ATG
Reverse primer TmiS0R	5' GCA ACA TAA GTG TAC AGC TG
Primers for amplification of the S1 tandem repeat region	
Forward primer TmiS1F	5' CAG CTG TAC ACT TAT GTT GC
Reverse primer TmiS1R	5' TCG TTC GCC TCG AAG ACG CAC
Primers for amplification of the S2 tandem repeat region	
Forward primer TmiS2F	5' GAC CTT CAT TCT AGC TAT G
Reverse primer TmiS2R	5' CCT ATC GTA CGA GAA CGT TAG

This was followed by 20 cycles using exactly the same thermal profile, but with an additional 20 s of extension time on each repeated cycle. A terminal extension of 4 min at 68 °C completed the reaction.

The NTS PCR product was purified by gel electrophoresis, and subcloned into the vector pGEM-T Easy (Promega). The NTS from strain 2111 was selected for sequencing based on the low-complexity rDNA pattern (type P-1) shown by this isolate in a previous study (Mochizuki *et al.*, 2003). The NTS was sequenced on the sense and anti-sense strands using a primer-walking strategy. Reactions were carried out using dye terminator chemistry on an Applied Biosystems 377 automated sequencer. Repetitive tracts of DNA were initially located using a nucleic acid dot-matrix self-comparison program (<http://arbl.cvmbs.colostate.edu/molkit/dnadot/>), and verified manually. Secondary structure predictions for sequence at the 3' end of the large subunit (LSU) rRNA gene were carried out using the RNAfold program (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>).

PCR amplification of the subrepeat regions S0, S1 and S2.

A single generic PCR reaction mixture and thermal profile was developed to allow amplification of all three polymorphic loci using the same conditions. PCR primers were designed for the specific amplification of each of the three loci containing repeats TmiS0, TmiS1 and TmiS2 (Table 2). The PCR mixture contained 1× reaction buffer (Promega), 1.5 mM magnesium chloride, 20 pmol each primer, 0.2 mM each dNTP and 1.25 units *Taq* polymerase (Promega), in a total volume of 49 µl. One microlitre of genomic DNA template was added per reaction containing >10⁴ copies of the target sequence (~20 ng per reaction). Thermal cycling conditions were an initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 90 s. A terminal extension of 5 min at 72 °C completed the reaction. Polymorphisms between PCR products from each locus were identified by comparative gel electrophoresis

RESULTS AND DISCUSSION

Characterization of the *T. mentagrophytes* var. *interdigitale* NTS region

The NTS region was 3322 bp in length, and bounded upstream by the 3' terminus of the LSU rDNA gene, and downstream by the 5' end of the small subunit (SSU) rDNA gene. The end of the LSU rDNA gene was predicted by

comparison with sequence from the experimentally determined 3' terminus of the *Candida albicans* 25S gene. However, adjacent to and downstream from this predicted terminus was a 59 bp sequence that was highly conserved between *T. rubrum*, *Trichophyton violaceum*, *Trichophyton tonsurans* and *T. mentagrophytes* var. *interdigitale*. The NTS sequences of all four species diverged significantly immediately after this region. The conserved sequence formed part of the 3' external transcribed spacer (ETS), and secondary structure predictions indicated that part of this region could fold into a stable stem-loop structure. A similar structure in *Saccharomyces cerevisiae* functions in the processing of the pre-rRNA transcript and maturation of the LSU RNA (Allmang & Tollervy, 1998). An illustration of the conserved region is available in Supplementary Fig. S1 in JMM Online.

Identification of SREs

Dot-matrix self-comparison of the NTS sequence from isolate 2111 identified three separate regions containing repetitive elements. Each individual repeat unit was identified using co-ordinates from the dot matrix, and also by comparison with known SREs from *T. rubrum* (Jackson *et al.*, 2000) and *T. tonsurans* (Gaedigk *et al.*, 2003). The repeats in each of the three regions varied markedly in number, length and sequence homology. The first region, named TmiS0, contained eight copies of a short, highly degenerate repeat that varied in length from 32 to 43 residues, with an interruption of 20 bp in the fourth repeat (Fig. 1). There were no counterparts of the TmiS0 elements in either *T. rubrum* or *T. tonsurans*.

The second region, TmiS1, contained two much larger 300 bp repeats, which were homologous to the interrupted variable internal repeats (VIRs) identified in *T. tonsurans* (Gaedigk *et al.*, 2003). The TmiS1 elements were also located in a very similar region of the NTS to the large TrS1 repeats of *T. rubrum* (Jackson *et al.*, 2000), but were substantially longer (300 versus 200 bp) and bore limited sequence

Fig. 1. Diagrammatic representation of the NTS region of *T. mentagrophytes* var. *interdigitale* isolate 2111. The approximate positions of and exact repeat size and copy number for the TmiS0, TmiS1 and TmiS2 SRE loci are indicated.

homology to them. Partial or truncated copies of TrS1 exist in the NTS of *T. rubrum*, and two similarly attenuated copies of the TmiS1 SREs were identified adjacent to and downstream of the main cluster of TmiS1 elements (Fig. 1). Homologues of these partial elements have been categorized as discrete repetitive units in *T. tonsurans* (Gaedigk *et al.*, 2003), but sequence alignments suggest that in *T. mentagrophytes* var. *interdigitale*, they are specifically truncated forms of entire TmiS1 repeats.

A limited number of VIRs from *T. tonsurans* have been found to contain an 'interruption' sequence (Gaedigk *et al.*, 2003). When multiple TmiS1 elements were sequenced from different strains of *T. mentagrophytes* var. *interdigitale*, this 'interruption' was present in every copy ($n=12$), and thus formed an integral part of the TmiS1 repeat structure in this species.

The third region, named TmiS2, contained four full-length repeats of between 85 and 89 bp, and two partial copies of 70 and 66 bp, respectively. The TmiS2 repeats showed substantial positional and primary sequence conservation with the TrS2 repeat region of *T. rubrum* and TvS2 of *T. violaceum*, in contrast to *T. tonsurans*, which contains no repetitive elements at this location. A comparison of the S2 repeats in these species is available in Supplementary Fig. S2 in JMM Online.

Strain identification by PCR fingerprinting of the three Tmi subrepeat loci

Amplification products from each of the three loci showed different types of strain-specific polymorphisms (Fig. 2a–c). The least polymorphic locus was TmiS2, for which 37/42 isolates (88 %) had a single-banded PCR product of 684 bp. This was designated pattern type II. Two isolates (EP0843 and EK1471) produced a smaller fragment of approximately 600 bp (type I), which was also amplified from the

teleomorph species *Arthroderma vanbreuseghemii*. Two further isolates (EF2608 and EP1174) produced progressively larger fragments of approximately 920 and 1000 bp, respectively, designated types III and IV. The TmiS2 pattern types I–IV are illustrated in Fig. 2(c). The size of the TmiS2 elements was approximately 85 bp, and hence the type I pattern may represent five repeats; type II, six repeats; type III, ten repeats and type VI, 11 repeats.

The TmiS0 locus showed a higher degree of variability (Fig. 2a), with ten different PCR types identified from the 42 isolates. However, a majority of strains (27/42; 64 %) again showed one common pattern type (type D), represented by a single 800 bp PCR fragment. The nine other variable types, designated A–C and E–J, were identified either from low numbers of isolates or in single strains only (Table 1). The first six types (A–F) had a single band of variable size, but four isolates (types G–J) had complex patterns containing multiple bands (Fig. 2a). Two isolates from India (SM8774 and SM8779) failed to amplify with these primers. A characteristic low-molecular-mass band of approximately 450 bp was amplified from the teleomorph *A. vanbreuseghemii* (lane B, Fig. 2a). Preliminary sequence data for some of the PCR fragments from types A–E indicate that a combination of repeat element multiplications and insertion/deletion events is responsible for the polymorphisms observed at this locus.

The TmiS1 region showed the greatest variation, but as with the other loci, a single PCR pattern predominated. This pattern, designated type 2, was represented by a 1175 bp PCR fragment containing two copies of the TmiS1 SRE, and was present in 23/42 isolates (55 %). Five simple pattern types (types 1–5) were observed with incremental size variations corresponding to full copies of the TmiS1 element. Five multiple-banded, complex PCR profiles (types 6–10) were identified for eleven isolates (lanes

Fig. 2. PCR fingerprint patterns determined for 42 isolates of *T. mentagrophytes* var. *interdigitale* at the TmiS0 (a), TmiS1 (b) and TmiS2 (c) subrepeat loci.

6–10, Fig. 2b). Two isolates from India (SM8774 and SM8779) again failed to amplify with these primers. A unique, complex pattern (type 0; Fig. 2b, lane 0) was amplified from the TmiS1 locus of *A. vanbreuseghemii*.

Pattern variability at each individual repeat locus appeared to occur independently of the other two. By combining the PCR profiles, a total of 19 PCR types were recognized from the 42 isolates (Table 3), with a further unique type for *A. vanbreuseghemii* (pattern 20, type B 0 1; Table 3).

Table 3. Combined S0, S1 and S2 fingerprint profiles for 19 PCR types of *T. mentagrophytes* var. *interdigitale*

Forty-two isolates were assayed, two of which failed to amplify with the S0 and S1 primers. The profile of one isolate of *A. vanbreuseghemii* is included in the results (marked with an asterisk).

Number	PCR type	No. isolates (percentage of total)
1	D 2 II	16 (39)
2	D 7 II	3 (7)
4	C 2 II	3 (7)
3	D 6 II	2 (5)
5	F 2 II	2 (5)
6	F 1 II	1 (2.4)
7	D 3 II	1 (2.4)
8	D 4 II	1 (2.4)
9	D 6 II	1 (2.4)
10	D 9 II	1 (2.4)
11	D 7 IV	1 (2.4)
12	G 2 II	1 (2.4)
13	G 1 II	1 (2.4)
14	E 8 III	1 (2.4)
15	H 10 II	1 (2.4)
16	I 7 IA	1 (2.4)
17	J 5 II	1 (2.4)
18	C 2 I	1 (2.4)
19	A 1 I	1 (2.4)
20*	B 0 1	1 (2.4)
Total	—	41 (100)

Reproducibility was high, with DNA extracted from the same strain by different investigators producing identical PCR profiles. Two isolates from the Indian subcontinent produced fragments with the TmiS2 primers, but could not be typed from the S0 and S1 regions. Phenotypically, these isolates were *T. mentagrophytes* var. *interdigitale*, and showed ITS restriction profiles characteristic of this species. However, isolates previously identified as *T. mentagrophytes* var. *mentagrophytes* (Mochizuki *et al.*, 2003) which were from non-foot sites with patients reporting animal contact as a likely causal factor, also produced an S2 product alone when amplified with the three sets of fingerprinting primers (data not shown). We are therefore further investigating the molecular identity of the Indian strains by sequence analysis of the ITS and NTS regions.

A. vanbreuseghemii is the teleomorphic species from which the sexually degenerate anamorph *T. mentagrophytes* var. *interdigitale* has evolved (Gräser *et al.*, 1999a). The teleomorph contains low copy numbers of both the TmiS0 and TmiS2 repeats (four and five, respectively) relative to *T. mentagrophytes* var. *interdigitale*, and the high repeat copy numbers in the latter therefore appear to have arisen during the course of its host adaptation and divergent evolution from *A. vanbreuseghemii*. Interestingly, although the closely related species *T. tonsurans* has counterparts of the TmiS1 repeats, it entirely lacks any SREs at the TmiS0

and TmiS2 positions in the NTS (Gaedigk *et al.*, 2003). Although the TmiS1 SREs are very similar to the VIRs of *T. tonsurans*, the large repeats (the TrS1 elements) that exist at a comparable location in the NTS of *T. rubrum* bear limited sequence homology to either the TmiS1 or VIR elements. This particular region of the NTS may represent a 'repetition hotspot', as similar types of unrelated repeats appear to have arisen independently in different dermatophyte species. Palindromic sequences are a feature of all these large repeats, and may be involved in a saltatory mechanism of repeat generation. Multiple copies of the TmiS1 elements show high sequence conservation within and between isolates, and appear to be generated by duplicative processes involving the second and subsequent S1 elements, but not the first or last.

In contrast to the divergence of the TmiS1 and TrS1 repeats, the TrS2 elements in *T. rubrum* have close sequence homology to the corresponding TmiS2 repeats in *T. mentagrophytes* var. *interdigitale*, and these conserved repetitive regions, proximal to the SSU gene, may have a role in the regulation of rDNA transcription.

Pattern distributions among clinical sites/countries

There are remarkable parallels between the distribution of PCR types in *T. mentagrophytes* var. *interdigitale* and of those in *T. tonsurans* and *T. rubrum*. In all three species, a majority of isolates belong to only one PCR type, and this type is characterized by having either one or two copies of the main repeat element. In *T. rubrum* and *T. mentagrophytes* var. *interdigitale*, the repeat locus closest to the SSU rRNA gene (TmiS2, TrS2) shows much less interstrain polymorphism than the second, larger locus (TmiS1, TrS1). The predominance of a single PCR type, and its occurrence in different geographic locations, imply clonality in all these species. In the current study, no clear associations existed between the PCR type and the geographic origin or clinical site of isolates, but a much larger and better-documented sample base is required in order to identify any such links.

Conclusion

Molecular studies have demonstrated that, as with *T. rubrum* (Gräser *et al.*, 1999b), there is a lack of differentiation between strains of *T. mentagrophytes* var. *interdigitale* at the genetic level (Mochizuki *et al.*, 1996). Adapted strains may have spread throughout the human population very rapidly (Charif & Elewski, 1997), and this recent emergence or 'epidemic' spread of a limited number of strain types has generated the pattern distributions we observe here. It is possible that this explosive increase in dermatophyte population size has influenced the accumulation of polymorphisms in specific genetic markers, such as the rRNA subrepeat elements reported here, since the copy number of such tandemly repetitive regions is regulated by events occurring at mitosis, such as unequal crossover (Szostak & Wu, 1980). These markers, and other mini- and

microsatellites, currently represent the most effective, and often the only practical means, for the molecular subtyping of anthropophilic dermatophytes (Jackson *et al.*, 1999, 2000; Gaedigk *et al.*, 2003; Mochizuki *et al.*, 2003; Ohst *et al.*, 2004). Our report of a rapid, simple and discriminatory method for subtyping *T. mentagrophytes* var. *interdigitale* will provide a valuable resource for future investigations into the epidemiology and pathogenesis of this species.

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REFERENCES

- Allmang, C. & Tollervey, D. (1998). The role of the 3' external transcribed spacer in yeast pre-rRNA processing. *J Mol Biol* **278**, 67–78.
- Charif, M. A. & Elewski, B. (1997). A historical perspective on onychomycosis. *Dermatol Ther* **3**, 43–45.
- Drake, L. A., Dinehart, S. M., Farmer, E. R. & 15 other authors (1996). Guidelines of care for superficial mycotic infections of the skin: tinea corporis, tinea cruris, tinea faciei, tinea manuum, and tinea pedis. *J Am Acad Dermatol* **34**, 282–286.
- Gaedigk, A., Gaedigk, R. & Abdel-Rahman, S. M. (2003). Genetic heterogeneity in the rRNA gene locus of *Trichophyton tonsurans*. *J Clin Microbiol* **41**, 5478–5487.
- Gräser, Y., Kuijpers, A. F. A., Presber, W. & De Hoog, G. S. (1999a). Molecular taxonomy of *Trichophyton mentagrophytes* and *T. tonsurans*. *Med Mycol* **37**, 315–330.
- Gräser, Y., Kuhnisch, J. & Presber, W. (1999b). Molecular markers reveal exclusively clonal reproduction in *Trichophyton rubrum*. *J Clin Microbiol* **37**, 3713–3717.
- Jackson, C. J., Barton, R. C. & Evans, E. G. V. (1999). Species identification and strain differentiation of dermatophyte fungi by analysis of ribosomal DNA intergenic spacer regions. *J Clin Microbiol* **37**, 931–936.
- Jackson, C. J., Barton, R. C., Kelly, S. L. & Evans, E. G. V. (2000). Strain identification of *Trichophyton rubrum* by specific amplification of subrepeat elements in the ribosomal DNA nontranscribed spacer. *J Clin Microbiol* **38**, 4527–4534.
- Mochizuki, T., Watanabe, S. & Uehara, M. (1996). Genetic homogeneity of *Trichophyton mentagrophytes* var. *interdigitale* isolated from geographically distant regions. *J Med Vet Mycol* **34**, 139–143.
- Mochizuki, T., Ishizaki, H., Barton, R. C., Moore, M. K., Jackson, C. J., Kelly, S. L. & Evans, E. G. (2003). Restriction fragment length polymorphism analysis of ribosomal DNA intergenic regions is useful for differentiating strains of *Trichophyton mentagrophytes*. *J Clin Microbiol* **41**, 4583–4588.
- Ohst, T., de Hoog, S., Presber, W., Stavrakieva, V. & Gräser, Y. (2004). Origins of microsatellite diversity in the *Trichophyton rubrum*–*T. violaceum* clade (dermatophytes). *J Clin Microbiol* **42**, 4444–4448.
- Smith, E. S., Fleischer, A. B. & Feldman, S. R. (1998). Nondermatologists are more likely than dermatologists to prescribe antifungal/corticosteroid products: an analysis of office visits for cutaneous fungal infections, 1990–1994. *J Am Acad Dermatol* **39**, 43–47.

Szostak, J. & Wu, R. (1980). Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae*. *Nature* **284**, 426–430.

Takiuchi, I., Morishita, N., Hamaguchi, T., Ninomiya, J. & Higuchi, R. (2005). Treatment outcome and relapse with short-term oral terbinafine (250 mg day⁻¹) in tinea pedis. *Nippon Ishinkin Gakkai Zasshi* **46**, 285–289.

Tosti, A., Piraccini, B. M., Stinchi, C. & Colombo, M. D. (1998). Relapses of onychomycosis after successful treatment with systemic antifungals: a three-year follow-up. *Dermatology* **197**, 162–166.

White, T. J., Bruns, T. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a Guide to Methods and Applications. Part 3: Genetics and Evolution*, Chapter 38, pp. 315–322. Edited by M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White. London: Academic Press.

Zaias, N. & Rebell, G. (2003). Clinical and mycological status of the *Trichophyton mentagrophytes* (*interdigitale*) syndrome of chronic dermatophytosis of the skin and nails. *Int J Dermatol* **42**, 779–788.