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Mating Genes of the *Trichophyton mentagrophytes* Complex

Rui Kano · Masako Kawasaki · Takashi Mochizuki · Masatarou Hiruma · Atsuhiko Hasegawa

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Abstract The mating type (−)-specific gene of the alpha-box and the mating type (+)-specific gene of the high-mobility group (HMG) DNA-binding domain were confirmed in zoophilic dematophytes of *Arthroderma simii* and *A. vanbreuseghemii*. The sequence of the alpha-box gene was 1,375 bp, containing 2 exons (from 172 to 463 bp and from 513 to 1,375 bp) in the *A. simii* (−) mating type strain and 1,380 bp, containing 2 exons (from 177 to 468 bp and from 518 to 1,380 bp) in the *A. vanbreuseghemii* (−) mating type strain. The sequence of the *HMG* gene was 1,871 bp, containing 2 exons (from 181 to 362 bp and from 426 to 1,440 bp, coding a protein of 398 amino acids) in the *A. simii* (+) mating type strain and 1,811 bp containing 2 exons (from 158 to 339 bp and from 403 to 1,381 bp, coding a protein of 386 amino acids) in the *A. vanbreuseghemii* (+) mating type strain. Of 15 animal isolates and 72 human isolates examined, the alpha-box gene was detected in five of the animal isolates and in none of the human isolates, while the *HMG* gene was detected in the other 10 of the animal isolates and in all of the human isolates. Phylogenetic analysis of the alpha-box and *HMG* genes of *Trichophyton mentagrophytes* complex strains and the *Microsporum gypseum* strain revealed that these strains were divided into 4 clusters; the first cluster consisting of *A. vanbreuseghemii* and the isolates from animals and humans, the second cluster consisting of *A. simii*, the third cluster consisting of *A. benhamiae* and the fourth cluster consisting of *M. gypseum*. These results indicate that anthropophilic *T. mentagrophytes* evolved from the *A. vanbreuseghemii* (+) mating strain.

Keywords Anthropophilic · *Arthroderma vanbreuseghemii* · Alpha-box · HMG · Mating type · *Trichophyton interdigitale*

Present Address:
R. Kano (✉)
Department of Pathobiology, Nihon University School of Veterinary Medicine, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan
e-mail: kano@brs.nihon-u.ac.jp

M. Kawasaki · T. Mochizuki
Department of Dermatology, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan

M. Hiruma
Department of Dermatology and Allergology, Juntendo University Nerima Hospital, 3-1-10, Takanodai, Nerima, Tokyo 177-0033, Japan

A. Hasegawa
Teikyo University Institute of Medical Mycology, 539 Otsuka, Hachioji, Tokyo 192-0395, Japan

Introduction

Trichophyton mentagrophytes is epidemiologically divided into two distinct forms, zoophilic and anthropophilic [1]. The zoophilic isolates of *T. mentagrophytes*

have been generally identified by morphological and biochemical examination as well as through mating experiments. The confirmed teleomorphs of the zoophilic isolates of the *T. mentagrophytes* complex are *Arthroderma benhamiae*, *A. simii* and *A. vanbreuseghemii* [1–4]. On the other hand, no teleomorph has been identified in an anthropophilic isolate of *T. mentagrophytes*, such as *T. mentagrophytes* var. *interdigitale* (*T. interdigitale*), or in the other anthropophilic strains, such as *T. rubrum.* However, *T. interdigitale* generally reacts as a (+) type by producing pseudoascocarps when paired with tester strains of *A. benhamiae*, *A. vanbreuseghemii* or *A. simii* [1–4], suggesting evolution from geophilic strains to the zoophilic strains and eventually to the anthropophilic strains, including *T. interdigitale* strains [1]. Heidemann et al. [5] re-classified the *T. mentagrophytes* complex into 4 groups and established the groups of zoophilic and anthropophilic strains of *T. interdigitale* (related to *A. vanbreuseghemii*) by internal transcribed spacer (ITS) region analysis. However, the mechanism by which *T. interdigitale* lost the ability to mate in the process of evolving to being anthropophilic is unknown.

In a previous study, we identified the mating type (−)-specific gene of the alpha-box and the mating type (+)-specific gene of the high-mobility group (HMG) DNA-binding domain in zoophilic dematophytes of *A. benhamiae* [6]. In the present study, these genes were examined in the clinical isolates of zoophilic and anthropophilic *T. mentagrophytes* as well as in zoophilic dematophytes of *A. simii* and *A. vanbreuseghemii* by PCR analysis.

Materials and Methods

Strains

The (+) and (−) mating type strains of *A. simii* and of *A. vanbreuseghemii* (+) and (−) were used in this study. The clinical isolates of *T. mentagrophytes*, 15 from animals and 72 from human tinea pedis, used are listed in Table 1.

Preparation of Genomic DNA

The mycelia were obtained by culturing isolates in Sabouraud's dextrose broth (1% peptone and 4% dextrose) at 27°C for 5 days. The mycelial cells of dermatophytes were collected by centrifugation at 1,500×*g* for 5 min and then frozen in liquid nitrogen and homogenized. They were then lysed in a lysis buffer containing 1 mg of zymolyase-100T/ml (Takara Bio Company, Kyoto, Japan), 0.1 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 10 mM Tris hydrochloride (pH 8.0) and 0.3% 2-mercaptoethanol, at 37°C for 16 h. High molecular weight DNA was extracted from the mycelial cells by the phenol/chloroform method. DNA samples dissolved in TE buffer (10 mM Tris–HCl, pH 8.0 and 1 mM EDTA) were used for further analysis.

Alpha-Box Gene Sequencing

To clone the alpha-box gene from (−) mating type strains (VUT-77008 and VUT-77010), primers were prepared based on the conserved sequence of the alpha-box gene of the *A. benhamiae* (GenBank accession number, AB570254) [6]. The primers are listed in Table 2.

Thirty-five cycles of PCR amplification were performed under the following conditions: denaturation for 1 min at 94°C, primer annealing for 1 min at 55°C and polymerization for 2 min at 72°C in a total reaction volume of 30 µl of amplification mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM each deoxynucleoside triphosphate, 1.0 U Taq polymerase (Takara, Kyoto, Japan) and 0.5 µg of each primer.

The PCR products were purified by gel electrophoresis and cloned into the pCRII vector (Invitrogen, San Diego, CA, USA). The plasmid DNA from more than three clones of each species was extracted using the QIAGEN plasmid kit (QIAGEN, Vallencia, CA, USA) and separately sequenced by the dideoxy chain termination method using an ABI PRISM 310 Genetic Analyzer (PerkinElmer, Inc., Foster City, CA, USA).

HMG Gene Sequencing

To clone the *HMG* gene 2 (+) mating type strains (VUT-77007 and VUT-77009), primers were prepared based on the conserved sequence of the HMG gene of the *A. benhamiae* (GenBank accession number AB542198) [6]. The primers are listed in Table 2. The PCR products obtained were sequenced using the techniques mentioned above.

Table 1 Strains of *T. mentagrophytes* used this study

Species	Strain (mating type)	Origin	Homology[f] (%)
A. simii	VUT[a]-77009 (+)	IAM[b] 12708 = ATCC[c] 16447	
A. simii	VUT-77008 (−)	IAM 12709 = ATCC 16448	
A. vanbreuseghemii	VUT-77007 (+)	IAM 12710 = RV[d] 27960	
A. vanbreuseghemii	VUT-77010 (−)	IAM 12711 = RV 27961	
T. interdigitale	NUBS[e]-11001	Clinical isolate from dog	99*
T. interdigitale	NUBS-11002	Clinical isolate from dog	100*
T. interdigitale	NUBS-11003	Clinical isolate from cat	98*
T. interdigitale	NUBS-11004	Clinical isolate from hamster	100*
T. interdigitale	NUBS-11005	Clinical isolate from hamster	100*
T. interdigitale	NUBS-11006	Clinical isolate from hamster	100†
T. interdigitale	NUBS-11007	Clinical isolate from hamster	100†
T. interdigitale	NUBS-11008	Clinical isolate from hamster	100†
T. interdigitale	NUBS-11009	Clinical isolate from hamster	100†
T. interdigitale	NUBS-11010	Clinical isolate from dog	99†
T. interdigitale	NUBS-11011	Clinical isolate from dog	100†
T. interdigitale	NUBS-11012	Clinical isolate from dog	100†
T. interdigitale	NUBS-11013	Clinical isolate from dog	100†
T. interdigitale	NUBS-11014	Clinical isolate from deer	100†
T. interdigitale	NUBS-11015	Clinical isolate from rat	100†
T. interdigitale	NUBS-11016	Human tinea pedis	100†
T. interdigitale	NUBS-11017	Human tinea pedis	100†
T. interdigitale	NUBS-11018	Human tinea pedis	100†
T. interdigitale	NUBS-11019	Human tinea pedis	100†
T. interdigitale	NUBS-11020	Human tinea pedis	100†
T. interdigitale	NUBS-11021	Human tinea pedis	100†
T. interdigitale	NUBS-11022	Human tinea pedis	100†
T. interdigitale	NUBS-11023	Human tinea pedis	100†
T. interdigitale	NUBS-11024	Human tinea pedis	100†
T. interdigitale	NUBS-11025	Human tinea pedis	100†
T. interdigitale	NUBS-11026	Human tinea pedis	100†
T. interdigitale	NUBS-11027	Human tinea pedis	100†
T. interdigitale	NUBS-11028	Human tinea pedis	100†
T. interdigitale	NUBS-11029	Human tinea pedis	100†
T. interdigitale	NUBS-11030	Human tinea pedis	100†
T. interdigitale	NUBS-11031	Human tinea pedis	100†
T. interdigitale	NUBS-11032	Human tinea pedis	100†
T. interdigitale	NUBS-11033	Human tinea pedis	100†
T. interdigitale	NUBS-11034	Human tinea pedis	100†
T. interdigitale	KMU[f]-6408	Human tinea pedis	100†
T. interdigitale	KMU-6492	Human tinea pedis	100†
T. interdigitale	KMU-6493	Human tinea pedis	100†
T. interdigitale	KMU-6494	Human tinea pedis	100†
T. interdigitale	KMU-6495	Human tinea pedis	100†
T. interdigitale	KMU-6496	Human tinea pedis	100†
T. interdigitale	KMU-6497	Human tinea pedis	100†

Table 1 continued

Species	Strain (mating type)	Origin	Homology[f] (%)
T. interdigitale	KMU-6498	Human tinea pedis	100[†]
T. interdigitale	KMU-6499	Human tinea pedis	100[†]
T. interdigitale	KMU-6500	Human tinea pedis	100[†]
T. interdigitale	KMU-6503	Human tinea pedis	100[†]
T. interdigitale	KMU-6504	Human tinea pedis	100[†]
T. interdigitale	KMU-6505	Human tinea pedis	100[†]
T. interdigitale	KMU-6506	Human tinea pedis	100[†]
T. interdigitale	KMU-6507	Human tinea pedis	100[†]
T. interdigitale	KMU-6509	Human tinea pedis	100[†]
T. interdigitale	KMU-6511	Human tinea pedis	100[†]
T. interdigitale	KMU-6513	Human tinea pedis	100[†]
T. interdigitale	KMU-6514	Human tinea pedis	100[†]
T. interdigitale	KMU-6515	Human tinea pedis	100[†]
T. interdigitale	KMU-6516	Human tinea pedis	100[†]
T. interdigitale	KMU-6517	Human tinea pedis	100[†]
T. interdigitale	KMU-6518	Human tinea pedis	100[†]
T. interdigitale	KMU-6519	Human tinea pedis	100[†]
T. interdigitale	KMU-6520	Human tinea pedis	100[†]
T. interdigitale	KMU-6521	Human tinea pedis	100[†]
T. interdigitale	KMU-6522	Human tinea pedis	100[†]
T. interdigitale	KMU-6523	Human tinea pedis	100[†]
T. interdigitale	KMU-6524	Human tinea pedis	100[†]
T. interdigitale	KMU-6525	Human tinea pedis	100[†]
T. interdigitale	KMU-6526	Human tinea pedis	100[†]
T. interdigitale	KMU-6527	Human tinea pedis	100[†]
T. interdigitale	KMU-6530	Human tinea pedis	100[†]
T. interdigitale	KMU-6534	Human tinea pedis	100[†]
T. interdigitale	KMU-6535	Human tinea pedis	100[†]
T. interdigitale	KMU-6536	Human tinea pedis	100[†]
T. interdigitale	KMU-6537	Human tinea pedis	100[†]
T. interdigitale	KMU-6538	Human tinea pedis	100[†]
T. interdigitale	KMU-6539	Human tinea pedis	100[†]
T. interdigitale	KMU-6540	Human tinea pedis	100[†]
T. interdigitale	KMU-6541	Human tinea pedis	100[†]
T. interdigitale	KMU-6542	Human tinea pedis	100[†]
T. interdigitale	KMU-6543	Human tinea pedis	100[†]
T. interdigitale	KMU-6544	Human tinea pedis	100[†]
T. interdigitale	KMU-6545	Human tinea pedis	100[†]
T. interdigitale	KMU-6546	Human tinea pedis	100[†]
T. interdigitale	KMU-6547	Human tinea pedis	100[†]
T. interdigitale	KMU-6549	Human tinea pedis	100[†]
T. interdigitale	KMU-6550	Human tinea pedis	100[†]
T. interdigitale	KMU-6551	Human tinea pedis	100[†]
T. interdigitale	KMU-6552	Human tinea pedis	100[†]

Table 1 continued

Species	Strain (mating type)	Origin	Homology[f] (%)
T. interdigitale	KMU-6554	Human tinea pedis	100[†]

Sequence similarity with or alpha-box (GenBank accession no. AB605769)* or *HMG* (AB605767)[†] genes of *A. vanbreseghemii*

[a] *VUT* School of Veterinary Medicine, University of Tokyo

[b] *IAM* Institute of Applied Microbiology, University of Tokyo

[c] *ATCC* American Type Culture Collection

[d] *RV* Institute de Medecine Tropicale, Antwerp, Bergium

[e] *NUBS* Nihon University College of Bioresouce Sciences

[f] *KMU* Kanazawa Medical University

Table 2 Primer pairs used for the amplification of alpha-box gene and HMG gene of *T. mentagrophytes*

Sense primer	Sense primer sequence	Position	Reverse primer	Reverse primer sequence	Position	Amplicon	Target gene
TmMATa1S	CTCCCAGCCATCA ACAAAAC	1–20	TmMATa1R	GTTCACGCTGTCCTC GAATG	452–471	471	Alpha-box
TmMATa2S	GTTCTCGAGAGAA TGCCAAA	423–442	TmMATa2R	ACTCGACCTGCGTC ACGCAG	924–943	520	Alpha-box
TmMATa3S	CTTTCTGTTGACGA CATCGT	843–862	TmMATa3R	CCGTCAAACTGCGA GCAGTA	1,340–1,359	516	Alpha-box
TmHMG1	CCTCTTGATATCTG ATAAAC	1–20	TmHMG1R	GTTCACGCTGTCCTC GAATG	505–524	524	HMG gene
TmHMG2S	ATATGTATATCTCG GTGTCT	446–465	TmHMG2R	CAGATGGTTTTCTGG GAGCA	924–943	497	HMG gene
TmHMG3S	AACACCTGAGAC GAAAGCAC	863–882	TmHMG3R	CAGATGGTTTTCTGG GAGCA	1,290–1,309	446	HMG gene
TmHMG4S	CAAGGAGGAGAG TTCTTGTC	1,242–1,261	TmHMG4R	GGTTGAAGTACAAT CAAGTG	1,880–1,899	657	HMG gene
TmHMG5S	TCATACTGGTGTTT GGCGGG	1,526–1,545	TmHMG4R	GGTTGAAGTACAAT CAAGTG	1,880–1,899	373	HMG gene

PCR Analysis of the Alpha-Box and HMG Genes Specific to the Genomic DNA of Clinical Isolates of *T. mentagrophytes*

The primers TmMATa1S and TmMATa1R amplified a 471-bp fragment of the *A. vanbreuseghemii* alpha-box gene fragment. The primers TmHMG1S and TmHMG1R amplified a 524-bp fragment of the *A. vanbreuseghemii HMG* fragment.

Genomic DNA samples (100 ng) from the clinical isolates were amplified by PCR in a volume of 30 μl, using a reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM each deoxynucleoside triphosphate,

1.0 unit of *Taq* polymerase (Takara) and 0.5 μg of a pair of primers. Amplification was carried out over 35 cycles consisting of template denaturation (1 min, 94°C), primer annealing (1 min, 55°C) and polymerization (2 min, 72°C). The PCR products obtained were sequenced using the techniques mentioned above.

Phylogenetic Analysis of the Alpha-Box and HMG Genes

The DNA sequences were compared using Clustal W multiple sequence alignment programs [7], and a phylogenetic tree was constructed by the TREEVIEW displaying phylogenies program [8]. Bootstrap analysis was performed on 1,000 random samples and analyzed by the Clustal W programs [9].

Results

Alpha-Box Genes of *A. simii* and *A. vanbreuseghemii*

The sequence of the alpha-box gene of the *A. simii* (−) mating type was determined to be 1,375 bp, which contained 2 exons from 172 to 463 bp and from 513 to 1,375 bp, coding a protein of 384 amino acids, beginning with a putative initiating methionine (ATG).

The sequence of the alpha-box gene of the *A. vanbreuseghemii* (−) mating type was determined to be 1,380 bp, which contained 2 exons from 177 to 468 bp and from 518 to 1,380 bp, coding a protein of 384 amino acids, beginning with a putative initiating methionine (ATG).

The amino acid sequence of the *A. simii* alpha-box gene (GenBank accession number: AB605768) shared approximately 86.4, 92.6 and 78.1% sequence similarity with the *A. benhamiae*, *A. vanbreuseghemii* and *Microsporum gypseum* alpha-box amino acid sequences (GenBank accession number: AB570254, AB605769 and FJ798800) (Fig. 1), respectively, in the conserved region.

HMG Genes of *A. simii* and *A. vanbreuseghemii*

The sequence of the *HMG* gene of the *A. simii* (+) mating type was determined to be 1,871 bp, which contained 2 exons, from 181 to 362 bp and from 426 to 1,440 bp, coding a protein of 398 amino acids, beginning with a putative initiating methionine (ATG).

The sequence of the *HMG* gene of the *A. vanbreuseghemii* (+) mating type was determined to be 1,811 bp, which contained 2 exons, from 158 to 339 bp and from 403 to 1,381 bp, coding a protein of 386 amino acids, beginning with a putative initiating methionine (ATG). We determine the exons and introns of *HMG* gene *A. simii* and *A. vanbreuseghemii* by comparing with *HMG* gene *A. benhamiae* and *Microsporum gypseum HMG* (GenBank accession number: AB542198 and FJ798798).

The amino acid sequence of the *A. simii HMG* gene (GenBank accession number: AB605766) shared approximately 88.5, 88.1 and 65.3% sequence similarity with the *A. benhamiae*, *A. vanbreuseghemii* and *M. gypseum HMG* amino acid sequences (GenBank accession number: AB542198, AB605767 and FJ798798) (Fig. 2), respectively, in the conserved region.

PCR Detection of Alpha-Box and *HMG* Genes Specific to the Clinical Isolates of *T. mentagrophytes*

Of 15 animal isolates and 72 human isolates examined, the alpha-box gene was detected in 5 of the animal isolates and in none of the human isolates, while the *HMG* gene was detected in the other 10 of the animal isolates and in all of the human isolates.

Sequence similarity among clinical isolates of *T. mentagrophytes* and the type strains

The nucleotide sequence similarity was 99–100% among the alpha-box gene fragments from the 5 animal isolates and the regions of the type strain of *A. vanbreuseghemii* (−) mating type deposited in the database (DDBJ accession no. AB605769).

Similarly, the nucleotide sequence similarity was 99–100% among the HMG gene fragments from the 10 animal isolates and the regions of the type strain of *A. vanbreuseghemii* (+) mating type deposited in the database (DDBJ accession no. AB605769). Moreover, the nucleotide sequence similarity was 100% among the HMG gene fragments from the 72 human isolates and the *A. vanbreuseghemii* (+) mating type strain deposited in the database (DDBJ accession no. AB605769).

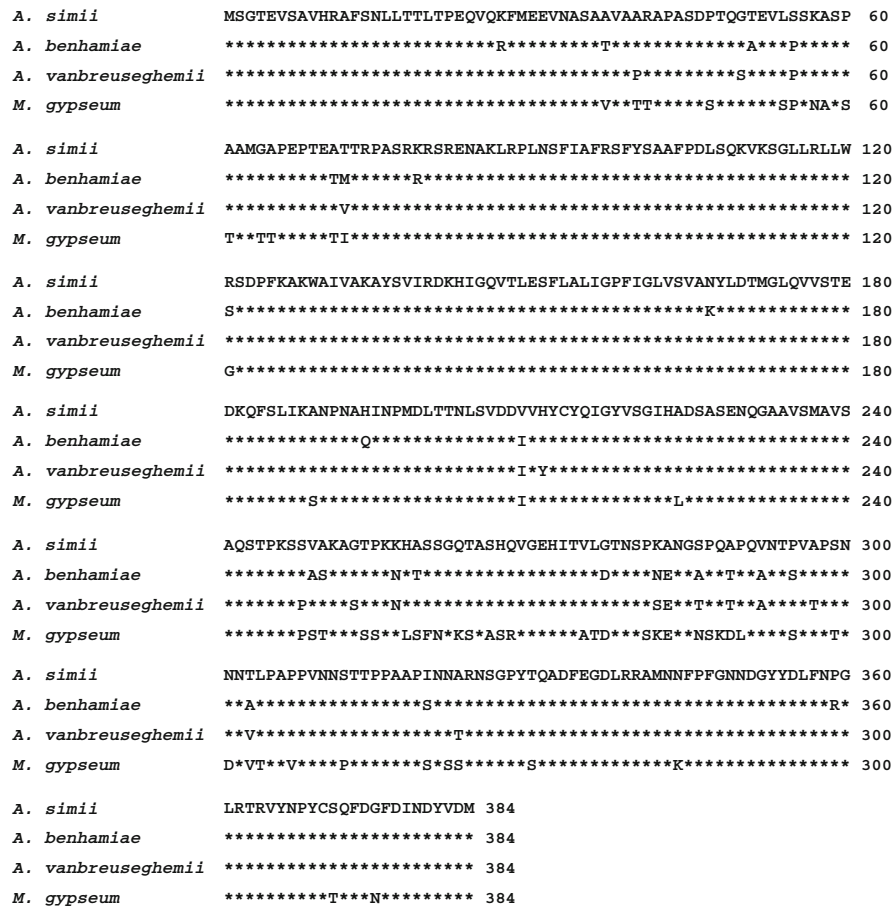

Fig. 1 Comparison of the homologous regions of the predicted protein sequences of the (−) mating type strains of the *A. simii* alpha-box (VUT-77008) (GenBank AB605768), *A. benhamiae* alpha-box (AB570254), *A. vanbreuseghemii* alpha-box (AB605769) and *M. gypseum* alpha-box (FJ798800). An *asterisk* indicates identity with the amino acid found in the *A. simii* alpha-box sequence

Phylogenetic Tree for the Alpha-Box and *HMG* Genes

Phylogenetic analysis of the alpha-box gene of *T. mentagrophytes* complex strains revealed that they were divided into four clusters; the first cluster consisting of *A. vanbreuseghemii* isolates and the five animal isolates, the second cluster consisting of *A. simii*, the third cluster consisting of *A. benhamiae* and the forth cluster consisting of *M. gypseum* (Fig. 3).

Phylogenetic analysis of the *HMG* gene of the *T. mentagrophytes* complex strains revealed that they were also divided into four clusters; the first cluster consisting of *A. vanbreuseghemii* and the 10 animal isolates and all 72 human isolates, the second cluster consisting of *A. benhamiae*, the third cluster consisting of *A. simii* and the forth cluster consisting of *M. gypseum* (Fig. 4).

Discussion

In this study, we identified the mating type (−)-specific gene of the alpha-box and the mating type (+)-specific gene of the HMG DNA-binding domain in *A. simii* and *A. vanbreuseghemii*. Kawasaki et al. [10] reported that these teleomorphs, *A. benhamiae*, *A. simii* and *A. vanbreuseghemii*, could mate among themselves and might be cospecific, although these species are from distant lineages. The amino acid sequence of the alpha-box gene in *A. simii* shared approximately 86.4, 92.6 and 78.1% similarity with those from *A. benhamiae*, *A. vanbreuseghemii* and *M. gypseum*, respectively, while the amino acid sequence of the HMG gene in *A. simii* shared approximately 88.5, 88.1 and 65.3% similarity with those in *A. benhamiae*, *A. vanbreuseghemii* and *M. gypseum*, respectively. These results indicate that more than 85% homology of alpha-box or HMG genes could be cospecific in dermatophytes.

Fig. 2 Comparison of the homologous regions of the predicted protein sequences of the (+) mating type strains of *A. simii HMG* (VUT-77009) (GenBank AB605766), *A. benhamiae HMG* (AB542198), *A. vanbreuseghemii HMG* (AB605767) and *M. gypseum HMG* (FJ798798) (GenBank AB605768). An *asterisk* indicates identity with the amino acid found in the *A. simii HMG* sequence. The *underlined amino acids* indicate the highly conserved *HMG* signature sequence "PRPPNAFILYR" and "PRKPSEKKRR" of filamentous ascomycetes (2)

PCR detected the *HMG* gene in all human isolates examined but did not detect the alpha-box gene in any human isolate. The *HMG* gene of anthropophilic isolates of *T. interdigitale* (*T. mentagropytes* from tinea pedis), with no mating ability, showed 100% homology with that of *A. vanbreuseghemii*. This result supports previous findings that *T. interdigitale* reacts as a (+) type by producing pseudoascocarps when paired with tester strains of *A. benhamiae*, *A. vanbreuseghemii* or *A. simii* [1].

Heidemann et al. [5] reported that the internal transcribed spacer (ITS) region of *T. interdigitale* is also genetically close to that of *T. mentagrophytes* from human tinea pedis and distinct from that of *T. mentagrophytes* from animals and human tinea corporis. Moreover, Symoens et al. [11] have shown that all the isolates from tinea pedis and tinea unguium identified as *T. interdigitale* based on ITS sequences mated with *A. vanbreuseghemii* tester strains, but had lost their ability to give fertile cleistothecia. Therefore, they suggested that *T. interdigitale* has to be considered as a humanized species derived from the sexual relative *A. vanbreuseghemii* [5, 11].

In the present study, the nucleotide sequences of the *HMG* gene fragments from 10 animal isolates with mating ability and 72 human isolates showed 99% (3–4 nucleotide changes in 420 bp) and 100% similarity, respectively, with those of the (+) mating type strain of *A. vanbreuseghemii* deposited in the database. The phylogenetic tree of the *HMG* gene indicates that the Japanese isolates of *T. mentagrophytes* from human tinea pedis are genetically close to animal isolates (Fig. 4). Therefore, these anthropophilic isolates of *T. mentagrophytes* may have derived from a

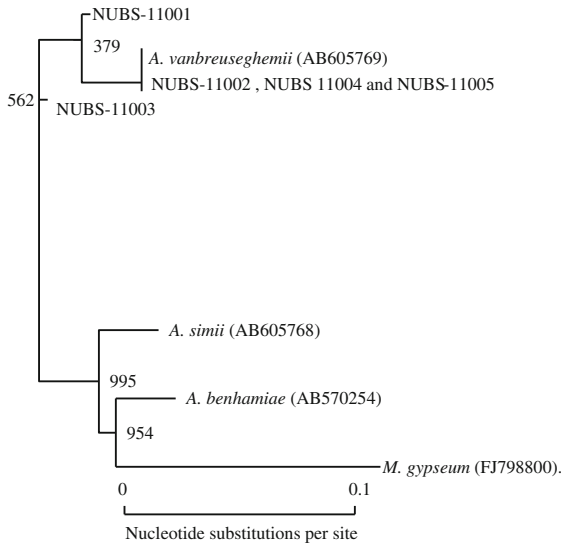

Fig. 3 A tree showing phylogenetic relationships among the obtained alpha-box gene sequences from dermatophyte species. *Numbers* at branches were determined by bootstrap analysis, indicating the times in 1,000 repeat sub-samples in monophylogenic grouping. *Bracket* indicates the DDBJ session number of alpha-box gene analysis of dermatophyte species

strain of the *A. vanbreuseghemii* (+) mating type and then widely developed.

A lack of sexual ability of fungi could be caused by (1) degeneration of sex genes, (2) increased heterozygosity among alleles, (3) a lack of transportable elements and (4) linkage disequilibria [11]. Moreover, genes at the mating locus and in the pheromone response pathway are highly conserved, even in apparently asexual fungi [11]. The present results regarding the anthropophilic isolates of *T. mentagrophytes* (*T. interdigitale*) from tinea pedis suggest that the sex genes might be degenerated and that heterozygosity among alleles might be increased, since the mating locus was highly conserved.

Evolutionary theory suggests that sex is expensive and inefficient, as it requires the formation of non-identical haploid gametes, which must fuse to regenerate a diploid [12]. Even in fungi, the need to maintain two mating partners reduces fitness [12]. However, sex emerged early in evolution, and most eukaryotes undergo a sexual cycle [12].

After *Homo sapiens* appeared on the earth around 200,000 years ago, the anthropophilic *T. mentagrophytes* would have evolved from the *A. vanbreuseghemii* (+) mating strain, losing its mating ability and proliferating on the host, while allowing recombination in stressful conditions [12]. Therefore, clinical isolates of anthropophilic *T. interdigitale* may be genetically close to the *A. vanbreuseghemii* (+) mating strain due to the lack of MAT.

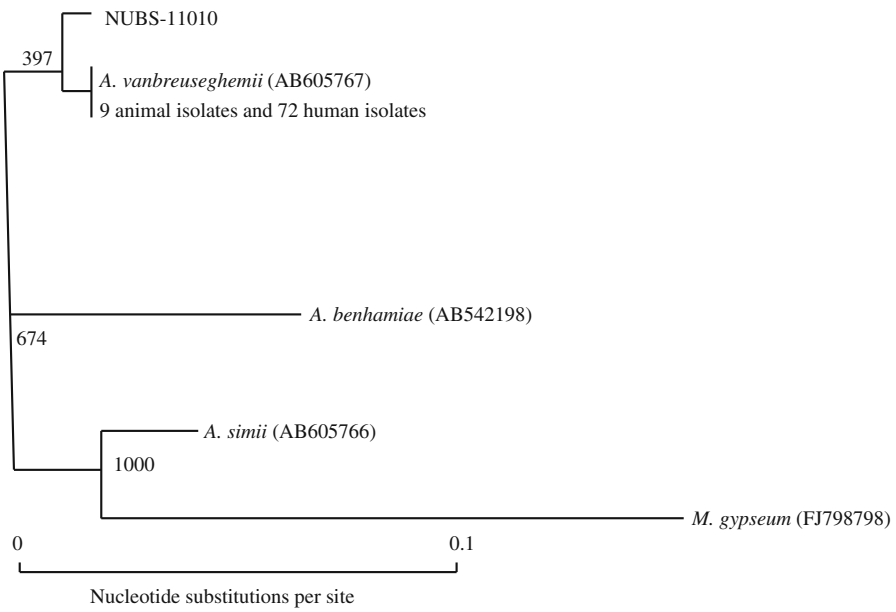

Fig. 4 A tree showing phylogenetic relationships among obtained *HMG* gene sequences of dermatophyte species. *Numbers* at branches were determined by bootstrap analysis, indicating the times in 1,000 repeat sub-samples in monophylogenic grouping. *Bracket* indicates the DDBJ session number of *HMG* gene analysis of dermatophyte species

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References

1. Kwon-Chung KJ, Bennett EJ. Dermatophytosis: medical mycology. Philadelphia: Lea & Febiger; 1992. p. 136–61.
2. Ajello L, Cheng S-L. The perfect state of *Trichophyton mentagrophytes*. Sabouraudia. 1967;4:230–4.
3. Stockdale MP, Mackenzie WRD, Austwick KCP. *Arthroderma simii* sp. nov., the perfect state of *Trichophyton simii* (Pinoy) comb. nov. Sabouraudia. 1967;4:112–23.
4. Takashio M. The *Trichophyton mentagrophytes* complex. In: Iwata K, editor. Recent advances in medical and veterinary mycology. Tokyo: University of Tokyo Press; 1977. p. 271–6.
5. Heidemann S, Monod M, Gräser Y. Signature polymorphisms in the internal transcribed spacer region relevant for the differentiation of zoophilic and anthropophilic strains of *Trichophyton interdigitale* and other species of *T. mentagrophytes* sensu lato. Br J Dermatol. 2010;62:282–95.
6. Kano R, Yamada T, Makimura K, Kawasaki M, Mochizuki T, Kamata H, Hasegawa A. *Arthroderma benhamiae* (The Teleomorph of *Trichophyton mentagrophytes*) mating type-specific genes. Mycopathologia. 2011;171:333–7.
7. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acid Res. 1994;22:4673–80.
8. Page RDM. TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci. 1996;12:357–8.
9. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39:783–91.
10. Kawasaki M, Anzawa K, Wakasa A, Takeda K, Mochizuki T, Ishizaki H, Hemashettar B. Matings among three teleomorphs of *Trichopyton mentagrophytes*. Nippon Ishinkin Gakkai Zasshi. 2010;51:143–52.
11. Symoens F, Jousson O, Planard C, Fratti M, Staib P, Mignon B, Monod M. Molecular analysis and mating behaviour of the *Trichophyton mentagrophytes* species complex. Int J Med Microbiol. 2011;301:260–6.
12. Butler G. Fungal sex and pathogenesis. Clin Microbiol Rev. 2010;23:140–59.