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Original Article

The Epidemiology and Mating Behavior of *Arthroderma benhamiae* var. *erinacei* in Household Four-toed Hedgehogs (*Atelerix albiventris*) in Japan

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Abstract

An epidemiological survey of *Trichophyton mentagrophytes* var. *erinacei* in the household hedgehog and other rodents was made between January 17, 2002 and February 28, 2002 in Japan. Quills and hairs were collected from sources identified via the internet. The fungus was isolated only from the quills of four-toed hedgehogs (7/18; 39%) from Kanto to Kyushu regions. Isolates were examined morphologically, physiologically and genetically, and identified as *T. mentagrophytes* var. *erinacei* anamorph. The isolates were also genetically compared with European hedgehog (*Erinaceus europeus*)-borne *T. mentagrophytes* var. *erinacei* and Kenyan hedgehog (*Aterelix albiventris*)-borne *Arthroderma benhamiae*, and their genotypes of the ITS1-5.8S-ITS2 rDNA were all identical. The isolates were crossed with *A. benhamiae* Americano-European race and African race, *A. vanbreuseghemii* and *A. simii*, with the result that they mated only with African race (+) or (−). Mating types of the isolates were (+) in 6 isolates and (−) in one. An intra-isolate mating between one of the 6 plus isolates and the minus one formed abundant mature gymnothesia, the mating type ratio of the F1 progeny was approximately 1:1, and the sib crossings of F1 progeny produced abundant fertile gymnothesia. The present study revealed that the intra-Japanese hedgehog-borne isolate crossing showed complete fertility and that the sexual degeneration pointed out by Takashio (Mycologia **71**: 968-976, 1979) did not exist. Two pairs of mating, (+) and (−) mating types of Japanese isolates with (−) and (+) tester strains of *A. benhamiae* African race formed less gymnothesia, mating type ratios were unbalanced, and sib crossings of F1 progeny produced small gymnothesia containing a low number of asci, pseudogymothesia, or none, respectively. These results show that *A. benhamiae* var. *erinacei*, the teleomorph of *T. mentagrophytes* var. *erinacei*, belongs to a different mating group (e.g. hedgehog race) than the Americano-European and African races in *A. benhamiae*.

Key words: *Arthroderma benhamiae* var. *erinacei*, epidemiology, four-toed hedgehog, mating, *Trichophyton mentagrophytes* var. *erinacei*.

Introduction

Trichophyton mentagrophytes var. *erinacei* is a zoonotic fungus affecting hedgehogs and also humans who come into contact with the animal[1]. We isolated *T. mentagrophytes* var. *erinacei* from a female household four-toed hedgehog (*Aterelix albiventris*) in Japan in 2001[2]. This alarmed us because hedgehogs are a popular household pet in Japan and this particular fungus spreads easily to humans. In the present study, we assessed the prevalence of *T. mentagrophytes* var. *erinacei* in household hedgehogs and other rodents in this country. The hedgehog-borne isolates were examined morphologically, physiologically and genetically, their teleomorphic status was assessed by mating tests, and results were compared with those of *T. mentagrophytes* var. *erinacei* isolated from European hedgehogs and infected humans in Europe and New Zealand, and those of *Arthroderma benhamiae* isolated from four-toed hedgehogs in Kenya[3].

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Table 1. The collection list of the quills and hairs studied.

Sample no.	Animal species	Sex	Age (Months)	Origin	Other accompanied animals	Residence
1*	FTH	F	24	PS-I	FTH, BS	Chiba
2*	FTH	−	9	PS-I	− or none	Chiba
3	FTH	F	12	PS	FTH, tropical fish	Tokyo
4	FTH	−	24	PS	Hamster	Fukushima
5	FTH	−	−	PS	Hamster	Ibaragi
6*	FTH	F	24	PS	None	Chiba
7	LEH	−	−	PS-I	Many**	Chiba
8	LEH	−	−	PS-I	Many**	Chiba
9	Tenrec	−	−	PS-I	Many**	Chiba
10	Tenrec	−	−	PS-I	Many**	Chiba
11	APP	−	−	PS-I	Many**	Chiba
12	FTH	−	4	−	Dog, rabbit, raccoon	Saitama
13*	FTH	−	6	PS	Tortoise, tropical fish	Ibaragi
14*	FTH	−	14	PS	FTH, dog	Okayama
15*	FTH	F	24	PS	−	Kanagawa
16	FTH	−	4	Internet	Hamster	Chiba
17	FTH	M	10	Home	FTH	Hiroshima
18	FTH	F	10	Home	FTH	Hiroshima
19	FTH	F	−	−	−	Saitama
20	LEH	−	48	Internet	Dog, rabbit, hamster, pigeon	Okayama
21	CPP	−	−	PS-I	−	Chiba
22	FTH	M	48	PS	Cat, pigeon, dog	Fukuoka
23*	FTH	F	18	PS	Cat, pigeon, dog	Fukuoka
24	ER-1	−	−	PS-I	Many**	Chiba
25	ER-2	−	−	PS-I	Many**	Chiba
26	FTH	M	24	PS	FTH, BS	Chiba
27	FTH	F	10	PS	FTH, BS	Chiba
28	BS	M	12	PS	FTH	Chiba

FTH; four-toed hedgehog, LEH; long-eared hedgehog, APP; African porcupine, CPP; Canadian porcupine, ER-1; European lemming, ER-2; European tiny harvest mouse, and BS; barking squirrel. M; male, and F; female. −; unknown. PS; pet shop, PS-I; pet shop dealing with imported individuals, Home; born at home, Internet; the internet shopping. *; fungus positive sample, and **; many animal species being kept at a pet shop in Chiba Prefecture.

Materials and methods

Samples: Quills and hairs from hedgehogs and rodents were collected between January 17, 2002, and February 28, 2002 from sources identified via the internet together with information of age, sex, origin and presence of other animals in the household. Sources were http://www2.jp-board.com/ii/bbs.cgi?room=hedgehog and http://res9.7777.net/bbs/hedgehoghouse/. The quill and hair samples were received by mail. Animals included 15 four-toed hedgehogs (*A. albiventris*), 3 long-eared hedgehogs (*Hemiechinas auritus*), 2 common tenrecs (*Tenrec ecaudatus*), 1 African crested porcupine (*Hystrix cristata*), 1 Canadian porcupine (*Erethizon dorsatum*), 1 European lemming (*Lagurus lagurus*), and 1 tiny harvest mouse (*Micromys minutus*). The statistic analysis of the present survey also included the first case of a four-toed hedgehog and its fellow resident animals, 2 other four-toed hedgehogs and a barking squirrel (*Cynomys ludovicianus*) reported previously[2] (Table 1).

Isolation: Quills and hairs were cultured at room temperature for 7 days on potato dextrose agar (PDA; Difco, Detroit, MI, USA) supplemented with 500 mg/*l* of cycloheximide and 50 mg/*l* of chloramphenicol. Whitish colonies grown on the root of the quills were inoculated onto PDA slants. For mating tests, both plus (+) and minus (−) mating types of *A. benhamiae* African race and *A. benhamiae* Americano-European race were used (Table 2). Morphological, physiological and genetic comparisons were made using 5 European and New Zealand *T. mentagrophytes* var. *erinacei* strains purchased from Centraalbureau voor Schimmelcultures (CBS), the Netherlands, 5 Kenyan *A. benhamiae* strains purchased from American Type Culture Collection (ATCC), USA and one European *T. mentagrophytes* var. *erinacei* maintained in the center as IFM 48154 (originally RV 28924) (Table 2).

Table 2. Mycological, physiological and genetical findings and mating behavior of the japanese hedgehog-borne, kenyan and European isolates

Isolate	IFM no.	Sample or strain no.	PDA obverse	PDA reverse	Circles	Urease	Max. Temp.	Pig. At 38°C	Mating type	Accession No.
Japanese isolates										
J1	IFM 50998	Sample 1	Powdery	Pale yellow	+	+	40°C	Brown	+	AB 078898
J2	IFM 51499	Sample 2	Powdery	Pale yellow	+	−	40°C	Brown	−	Identical*
J3	IFM 51500	Sample 6	Powdery	Lemon yellow	+	+	40°C	Brown	+	Identical
J4	IFM 51501	Sample 13	Powdery	Lemon yellow	+	+	41°C	Brown	+	Identical
J5	IFM 51502	Sample 14	Powdery	Pale yellow	+	+	41°C	Brown	+	Identical
J6	IFM 51503	Sample 15	Powdery	Pale yellow	+	+	41°C	Brown	+	Identical
J7	IFM 51504	Sample 23	Powdery	Pale yellow	+	+	41°C	Brown	+	Identical
Kenyan isolates of *A. benhamiae*										
K1	IFM 51419	ATCC 28433	Powdery	Pale yellow	+	+	40°C	Lemon yellow	−	Identical
K2	IFM 51420	ATCC 28434	Powdery	Pale yellow	+	+	40°C	Lemon yellow	−	Identical
K3	IFM 51421	ATCC 28435	Powdery	Pale yellow	+	+	40°C	Lemon yellow	−	Identical
K4	IFM 51422	ATCC 28436	Powdery	Pale yellow	+	+	40°C	Lemon yellow	−	Identical
K5	IFM 51423	ATCC 28437	Powdery	Pale yellow	+	+	40°C	Lemon yellow	−	Identical
European and New Zealand isolates of *T. mentagrophytes* var. *erinacei*										
E1	IFM 51378	CBS 511.73	Cottony	Lemon yellow	−	−	40°C	Lemon yellow	+	Identical
E2	IFM 51379	CBS 474.76	Cottony	Lemon yellow	−	−	40°C	Lemon yellow	+	Identical
E3	IFM 51380	CBS 344.79	Cottony	Lemon yellow	−	−	40°C	Lemon yellow	+	Identical
E4	IFM 51381	CBS 677.86	Powdery	Wine red	+	−	40°C	Lemon yellow	+	Identical
E5	IFM 51382	CBS 108.91	Cottony	Lemon yellow	−	+	40°C	Lemon yellow	+	Identical
E6	IFM 48154	RV 28924	Cottony	Lemon yellow	−	−	40°C	Lemon yellow	+	AB 078899
A. benhamiae (tester strains)										
AE(+)	IFM 48142	RV 26678	Cottony	Wine red	+	+	41°C	Brown	+	
AE(−)	IFM 48143	RV 26680	Cottony	Wine red	−	+	40°C	Brown	−	AF 170457 (12 base**)
Af(+)	IFM 48144	RV 30000	Cottony	Wine red	−	+	40°C	Brown	+	AF 170456 (25 base**)
Af(−)	IFM 48145	RV 30001	Powdery	Wine red	−	+	41°C	Brown	−	

IFM: Research center for Pathogenic Fungi & Microbial Toxicoses, Ciba University, Chiba. ATCC: American Type Culture Collection, Rockville, Maryland, USA. CBS: Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. RV: Institute de Medicine Tropicale, Antwerp, Belgium. Circles: Concentric circles on 1/10 diliuted salt-added Sabouraud's agar at 30°C. Mxs. temp.: Maximam growth temperature. Pig. At 38°C: Pigment production at 38°C. AE: *A. benhamiae* Americano-European race. Af: *A. benhamiae* African race.

*:Identical with AB 078898 and AB 078899 in ITS1-5.8S-ITS2 region. **: Difference from AB 078898 and AB 078899.

Mycological studies: Giant colonies cultured on Sabouraud's agar medium (glucose 2 %, peptone 1 %, agar 1.5%), PDA at 25°C and salt-added 1/10-diluted Sabouraud's agar medium (glucose 0.2%, Neopeptone Difco 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1%, KH_2PO_4 0.1%, Bacto agar 2 %) at 30°C were observed. As the present isolates formed concentric circles on the salt-added 1/10-diluted Sabouraud's agar, growth margins were marked on the reverse of the plates every 12 hours, and controls were left in continuous darkness. Transverse sections of the colonies fixed with lactophenol cotton blue were observed under a light microscope. Slide cultures were performed using PDA (25°C, 14 days).

Physiological studies: The urease activity on Christensen's urea agar slant (Eiken, Tokyo, Japan) was assessed at 25°C after 7 days. The maximum growth temperature and pigment production were evaluated at each degree from 37 to 42°C on PDA slant.

Genetic identification of *T. mentagrophytes* var. *erinacei*: Two isolates of *T. mentagrophytes* var. *erinacei* reported previously[2], 6 obtained for the present study, 5 of *T. mentagrophytes* var. *erinacei* purchased from CBS and 5 of Kenyan *A. benhamiae* purchased from ATCC were evaluated genetically (Table 2).

The DNA extraction and identification method was as previously reported[2]. Briefly, DNA was extracted by the benzyl chloride method[4] from isolates cultured on PDA at 25°C for 14 days, and the internal transcribed spacer region including 5.8S of ribosomal RNA gene (ITS1-5.8S-ITS2 rDNA) was detected by PCR with the primer pair ITS5 (5'-GGA AGT AAA AGT CGT AAC

AAG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The PCR products were labeled by ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4. The PCR-amplified sample was directly sequenced in both strands with a DNA sequencing kit (BigDye™ Terminator Cycle Sequencing Ready Reaction, ABI PRISM® Applied Biosystems, Tokyo, Japan) and sequenced on an ABI PRISM® 3100 Genetic Analyzer (AB Applied Biosystems, Hitachi). The nucleotide sequence data were analyzed with the genetic information processing software, GENETYX-MAC Ver. 10.1 (Software Development, Tokyo, Japan). A total of 591 bases were determined.

Mating tests: The present isolates were crossed with each tester strain of both (+) and (−) mating types of *A. benhamiae* African and Americano-European race, *A. simii* and *A. vanbreuseghemii*, and with each other. They were confronted on salt-added 1/10-diluted Sabouraud's agar medium and incubated at 25°C for 8 weeks.

Mating tests on F1 progeny: Ascospores produced by the mating of the two types of the *A. benhamiae* African race, that with hedgehog isolates or among the various hedgehog isolates, were spread over PDA plates to evaluate fertility. Briefly, a gymnothecium was cut under a magnifying stereoscope with two fine needles, and the asci were placed in 1.0 m*l* of sterile saline supplemented with 0.05% Tween 80 in a tube. After being vortexed and serially diluted with saline, 1.0 m*l* of each solution was spread onto a PDA plate and cultured at room temperature for 1-2 days. Forty single colonies of each were randomly selected from the mating of *A. benhamiae* (RV 30000) to *A. benhamiae* (RV 30001), IFM 50998 to *A. benhamiae* (RV 30001), IFM 51499 to *A. benhamiae* (RV 30000) and IFM 50998 to IFM 51499, respectively. The mating types of the F1 progeny were identified by the confrontation culture with the parents. The ratio of mating type (+) *versus* (−) was statistically analyzed by Chi square test.

Seven (+) and 7 (−) isolates from the three couples (IFM 50998×RV 30001, IFM 51499×RV 30000, IFM 50998×IFM 51499) of F1 progeny were randomly selected and sib crossings were performed in all possible combinations. The gymnothesia formations were observed after 8 weeks.

Results

Epidemiology: The samples were obtained from Fukushima, Ibaraki, Saitama, Tokyo, Chiba, Kanagawa, Okayama, Hiroshima, and Fukuoka. The animals infected with the fungus were distributed in Ibaraki, Chiba, Kanagawa, Okayama and Fukuoka Prefectures (Table 1).

Seven Japanese hedgehog-borne dermatophytes were isolated from 7 (sample nos. 1, 2, 6, 13, 14, 15 and 23) of the 18 four-toed hedgehogs (7/18; 39%) and registered to our center with their IFM numbers (IFM 50998, 51499, 51500, 51501, 51502, 51503 and 51504). There was no isolation of dermatophytes from other insectivoras or rodents (Table 1, 2).

Mycological findings: Results of the mycological studies performed on the Japanese hedgehog-borne isolates, Kenyan hedgehog-borne *A. benhamiae*, European hedgehog-borne *T. mentagrophytes* var. *erinacei* and tester strains of *A. benhamiae* are shown in Table 2.

Giant colonies of the hedgehog isolates on SDA showed a uniformly cottony elevation at the center; the surrounding area was white, powdery, and flattened. The reverse of each was uniformly cinnamon-colored. Giant colonies on PDA were

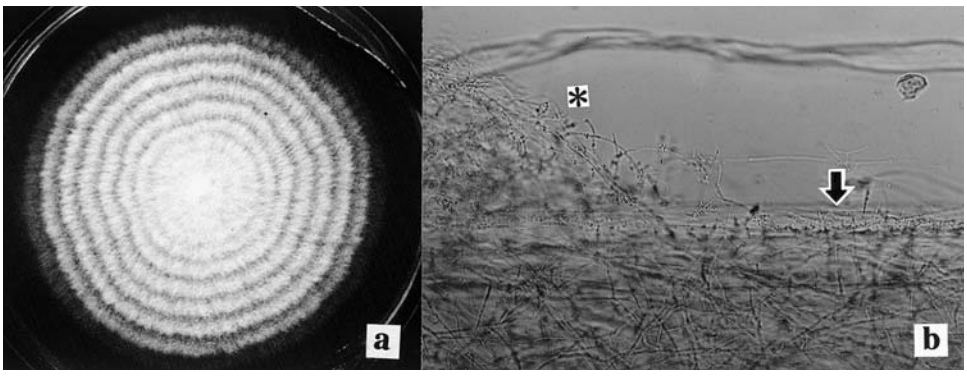

Fig. 1. Colony of Japanese hedgehog-borne isolate (IFM 50998) on a salt-added 1/10-diluted Sabouraud's agar plate at 30°C showed concentric circles every 24 hours (a), and its transverse section showed two structures of growth; aerial sporulations during the daytime (*) and expansion of mycelia during the nighttime (arrows) along with the agar plate (b).

cottony in the center part, and thinly lacy or powdery in the surrounding area. Color of the reverse was brilliant yellow or honey, and it diffused in a ring-like fashion. The obverse and reverse of the Kenyan isolates on PDA were the same texture and color as the Japanese isolates. All European isolates of *T. mentagrophytes* var. *erinacei* except one were cottony on the obverse and lemon yellow on the reverse. CBS 677.86 was powdery on the surface and orange yellow or sometimes wine red on the reverse.

On the salt-added 1/10-diluted Sabouraud's agar plates, all Japanese and Kenyan isolates produced thin colonies with concentric circles every 24 hours (Fig. 1a). This circle formation was observed despite the continuous darkness and constant temperature. Of the European isolates, only CBS 677.86 produced concentric circles. The formation of concentric circles was characteristic of the powdery form of *T. mentagrophytes* var. *erinacei*. Mycelial growth was predominant during the nighttime, and sporulation was active during the daytime. Two growth patterns, aerial sporulation and extension of mycelia along the agar surface were observed in the transverse section (Fig. 1b).

All tested hedgehog-borne isolates produced a few macroconidia and abundant microconidia in a pear-shaped or more elongated form arranged at right angles, mainly along the sides of the mycelium one-by-one in a slide culture using PDA. Many forms intermediate between micro- and macroconidia were also observed; spiral bodies were few. These morphological characteristics strongly suggested that all Japanese isolates were *T. mentagrophytes* var. *erinacei*.

Physiological findings: All but one Japanese hedgehog isolate (IFM 51499) showed urease activity; all of the Kenyan isolates also were urease-positive, while all European isolates except CBS 108.91 were negative for this activity. Of 18 examined, 12 were positive, 11 of which were from four-toed hedgehogs (Table 2).

Maximum growth temperature was 41°C for the tested strains of IFM 51501, 51502, 51503, 51504, *A. benhamiae* Americano-European race mating type (+) and *A. benhamiae* African race mating type (−), and 40°C for the others (Table 2).

Kenyan and European isolates produced a great deal of orange-yellow pigment on the PDA slant at 38°C, while, Japanese isolates produced brown pigment at this temperature (Table 2).

Genetic identification of *T. mentagrophytes* var. *erinacei*: The genotypes of the ITS1-5.8S-ITS2 rDNA were identical for all isolates tested. All sequences coincided with GenBank accession numbers AB078898 and AB078899. All the tested strains, including the 5 European *T. mentagrophytes* var. *erinacei* strains and the 5 Kenyan *A. benhamiae* strains were identified as *T. mentagrophytes* var. *erinacei* genotype.

Mating behavior: The Japanese isolates were crossed with *A. benhamiae* Americano-European race and African race, *A. vanbreuseghemii* and *A. simii*, with the result that they mated only with African race (+) or (−).

Mating types of the present isolates were (+) in 6 isolates and (−) in one. European isolates of *T. mentagrophytes* var. *erinacei* were all (+) mating type, while Kenyan isolates were all (−) mating type (Table 2).

Isolate IFM 51499 (−) mated successfully with other hedgehog isolates, and formed a broad band in the confronting area of both tested strains with abundant gymnothecia. Figure 2a shows that the mating of isolate IFM 50998 (+)

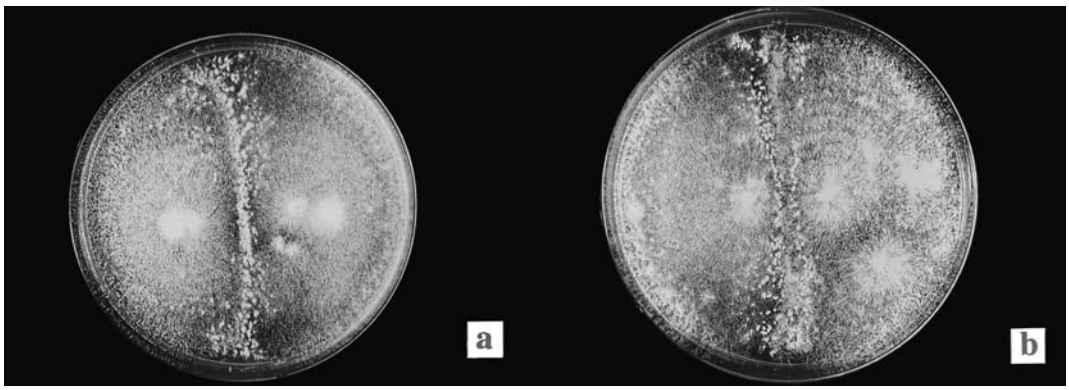

Fig. 2. a: Mating between Japanese isolate of *A. benhamiae* var. *erinacei* IFM 50998 (left) and 51499 (right) produced abundant gymnothesia along the confronting line.
b: Mating between F1 progeny of IFM 50998 × IFM 51499 (left) and the parent IFM 51499 (right) formed two bands with abundant gymnothesia.

Table 3. Ratio of mating type on F1 progeny by back crossing with the parents.

	F1 of Af (+)×Af (−)	F1 of J1 (+)×J2 (−)	F1 of J1 (+)×Af (−)*	F1 of J2 (+)×Af (+)
Mating type (+)	20 (50.0%)	22 (55.0%)	35 (87.5%)	14 (37.0%)
Mating type (−)	20 (50.0%)	18 (45.0%)	5 (12.5%)	24 (63.0%)
Total	40 (100%)	40 (100%)	40 (100%)	38 (100%)

F1: F1 progeny. J1: IFM 50998, J2: IFM 51499.
Af (+): *A. benhamiae* African race (IFM 48144), Af (−): *A. benhamiae* African race (IFM 48145).
*; a significant difference from the mating ratio of F1 of Af (+)×Af (−) and F1 of J1 (+)×J2 (−), $P<0.001$ by Chi square test.

to 51499 (−) produced abundant gymnothecia with many asci containing ascospores.

The gymnothecia between the present hedgehog-borne isolate and *A. benhamiae* (RV 30000 or RV 30001) formed a row along the *A. benhamiae* side. They produced small-sized gymnothesia containing a small number of ascospores and pseudogymnothesia.

Mating behavior of F1 progeny: Offspring by the four couples of mating were grown on PDA plates. The sexual ratio of F1 progeny of two couples of the mating, *A. benhamiae* RV 30000 (+) to *A. benhamiae* RV 30001 (−) and IFM 50998 (+) to IFM 51499 (−) was close to 1:1 (Fig. 2b); that of F1 progeny of the other two couples, IFM 50998 (+) to RV 30001 (−) and IFM 51499 (−) to RV 30000 (+) was unbalanced (Table 3). Comparison between the mating of *A. benhamiae* RV 30000 (+) with RV 30001 (−) and that of IFM 50998 (+) to IFM 51499 (−) was not significant by Chi square test. The mating ratio between RV 30001 (−) to IFM 50998 (+) was significantly different from *A. benhamiae* RV 30000 (+) with RV 30001 (−) and that of IFM 50998 (+) to IFM 51499 (−) ($P<0.001$).

All of the sib crossings of F1 progeny between IFM 50998 (+) and IFM 51499 (−) produced abundant fertile gymnothesia.

In contrast, the sib crossings of F1 progeny between IFM 50998 (+) and *A. benhamiae* RV 30001 (−) produced a small number of fertile gymnothesia and a large number of pseudogymnothesia or were infertile. Some of the sib crossings between IFM 51499 (−) and *A. benhamiae* RV 30000 (+) produced abundant small-sized gymnothesia containing few ascospores and pseudogymnothesia, and others produced few fertile gymnothesia, or none. The result of the examination, the teleomorph of *T. mentagrophytes* var. *erinacei* is *A. benhamiae* var. *erinacei*, and consists of a close but different mating group from the African race of *A. benhamiae*.

Discussion

In an epidemiological survey, *T. mentagrophytes* var. *erinacei* was reported in 52.5% of wild hedgehogs observed in New Zealand[5], and in 20～25% of wild hedgehogs in Britain[6]. The present study is the first epidemiological survey of household four-toed hedgehogs in Japan. It yielded an infection rate of 39% hedgehogs, and indicated that the dermatophytes may already have spread in the central and western parts of Japan.

It has been confirmed that Western European hedgehogs became wild in Tochigi, Kanagawa, and Nara after 1987, if not before[7]. The four-toed hedgehogs may also have adapted themselves to the natural environment of Japan, and *T. mentagrophytes* var. *erinacei* may have spread to the natural world.

Isolation of *T. mentagrophytes* var. *erinacei* from hedgehogs and humans in contact with the animals was first reported by Marples and Smith in New Zealand in 1960[1]. Since then, hundreds of zoonotic infections with *T. mentagrophytes* var. *erinacei* have been reported in Europe and New Zealand. Especially in humans, many cases of cutaneous ringworm, tinea barbae and kerion were reported[1, 8-11]. Although there have been no reports of human infection with this fungus in Japan, the high infection rate in household hedgehogs predicts that patients will be visiting their dermatologists in the near future. Infection with *A. benhamiae* var. *erinacei* could soon be listed as a new imported or naturalized mycosis.

Interestingly, most of the hedgehog owners kept other kinds of animals, such as dogs, raccoons, rabbits and hamsters, and imported-pet shops kept insectivoras like long-eared hedgehogs and tenrecs, but no dermatophytosis due to this fungus was found. In addition, there have been no reports of dermatophytosis in these animals except dogs[8], which directly or indirectly contact hedgehogs. This suggests that the fungus might be specific to four-toed and Western European hedgehogs. According to Kim *et al.*[12], DNA

patterns of *T. mentagrophytes* analyzed by the random amplified polymorphic DNA method indicated host species-specific band patterns. However, the specificity of *A. benhamiae* var. *erinacei* infection may also extend to humans and dogs.

Morphologies of Japanese and Kenyan hedgehog-born isolates cultured on PDA were nearly identical: cottony in the center and a thin lacy or powdery surface in the surrounding area. Most European isolates showed a cottony surface. The reverse color of the Japanese isolates varied from brilliant yellow to honey. According to Collinge *et al.*[13], the reverse color of Kenyan isolates was not brilliant at first but a bright yellow pigment developed later in some cultures. Some of our cultured Kenyan isolates also produced a yellow pigment. Most of the European isolates were brilliant yellow. Strain CBS 677.86, which produced a wine red pigment, changed to brilliant yellow under some culture conditions. The reverse color phenotype on PDA did not determine whether the Japanese hedgehog-borne isolates might have originated from those in Europe or Africa.

Our hedgehog isolates formed thin colonies with concentric circles on salt-added 1/10-diluted Sabouraud's agar plates maintained at a constant temperature (30°C) in continuous darkness, indicating a circadian rhythm for growth. A biological clock of the fungi has been reported only in *Neurospora crassa*[14]. *A. benhamiae* var. *erinacei* will be excellent material for research on the circadian rhythms of fungi.

According to Rush-Munro and Smith[15], the majority of *T. mentagrophytes* var. *erinacei* isolates are negative for urease activity. The present study, however, indicates that urease-positive isolates are predominant (12/18; 66.7%). On the basis of the mating type and of the urease activity, Takashio[16] divided the variety *erinacei* into three groups; (+) mating type and urease negative, (−) mating type and urease positive, and (+) mating type and urease positive. Strains from England and New Zealand corresponding to the type strain belonged to the first group, Kenyan isolates from four-toed hedgehogs (*Aterelix albiventris*) originally identified as *A. benhamiae* to the second group, and one strain from Belgium to the third one. Based on the grouping by Takashio, 6 of 7 (85.7%) Japanese isolates were in the third group. Interestingly, we found one Japanese isolate with (−) mating type and urease negative. This strain is not included in these three groups, and should be placed in a fourth group, which has not yet been found.

If the distribution of the mating type and urease activity depend on host species, whether *Erinaceus europeus* or *Atelerix albiventris*, the present survey should have yielded the entire second group, because the fungus positive hedgehogs were limited to four-toed hedgehogs, *Atelerix albiventris*. However, this study yielded 6 belonging to the third group, and one to the fourth group. These were characteristics of neither European hedgehog-borne isolates nor Kenyan hedgehog-borne isolates. Therefore, it is impossible to trace the import routes. However, *A. benhamiae* var. *erinacei* might have both mating types in Africa, because natural habitats of four-toed hedgehogs are limited to Central Africa.

T. mentagrophytes var. *erinacei* isolated in Europe and New Zealand was thought to have only the (+) mating type[17]. Therefore, there have been no reports on intra-hedgehog-borne isolate mating. We found the (+) and (−) mating types of Japanese hedgehog-borne isolates mated successfully with each other.

The (+): (−) mating type ratio in F1 progenies between Japanese isolates IFM 50998 (+) and IFM 51499 (−) was close to 1:1. These results indicate complete fertility among both (+) and (−) mating types of *T. mentagrophytes* from hedgehogs. The mating type ratio between the Japanese hedgehog-borne isolates and *A. benhamiae* African race was unbalanced. The data indicate an incomplete fertility among the *A. benhamiae* African race and the Japanese hedgehog isolates. This time only sprouted colonies of the F1 progeny population were collected; therefore, the population of mortal ascospores was not in the count. Nevertheless the unbalanced sex ratio may suggest some suppressive gene combinations resulting from the crosses between Japanese hedgehog-borne isolates and *A. benhamiae* African race.

The sib mating of F1 progeny between Japanese isolates of IFM 50998 (+) and IFM 51499 (−) formed a broad band or two bands with abundant fertile gymnothesia in the confronting area of both tested strains. On the other hand, most of the sib mating between Japanese hedgehog-isolates and *A. benhamiae* African race produced small-sized gymnothesia containing a small number of asci, pseudogymnothesia; a few of them were sterile. These results indicate that the coupling between hedgehog-borne isolates is a proper marriage and that between hedgehog-born isolates and *A. benhamiae* African race is an odd pairing.

Takashio[16] concluded that the teleomorph of *T. mentagrophytes* var. *erinacei* is *A. benhamiae* var. *erinacei* because of fertile crossing with *A.*

benhamiae African race, but var. *erinacei* is sexually partially degenerate. The present study revealed, however, that the intra-Japanese hedgehog-borne isolate crossing showed complete fertility and sexual degeneration did not exist. The partially sexual degeneration of *A. benhamiae* var. *erinacei* observed by Takashio might have been due to the mating var. *erinacei* to the African race.

According to Makimura *et al.*[18], comparison of the ITS1 rDNA between *A. benhamiae* complex and *T. mentagrophytes* var. *erinacei* showed an apparent variation. The present studies found that genotypes of the ITS1-5.8S-ITS2 rDNA were identical in all tested strains including 6 European hedgehog-borne *T. mentagrophytes* var. *erinacei* and 5 Kenyan four-toed hedgehog-borne *A. benhamiae*. Therefore, all isolates, even Kenyan isolates should be identified as *A. benhamiae* var. *erinacei* genotype.

In conclusion, the hedgehog-borne isolates belong to an independent variety, *erinacei*, in *A. benhamiae* because of morphological and physiological characteristics and the genetic uniformity, and may form a different mating group (e.g. hedgehog race) from Americano-European and African races of *A. benhamiae*.

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