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Mating experiment of *Microsporum canis* and *M. equinum* isolated from animals with *Nannizzia otae*

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Abstract

Fifty six isolates of *Microsporum canis* and 54 isolates of *M. equinum* obtained from animals in Japan were paired with either *Nannizzia otae* (+) or (–) tester strain. Of the 56 *M. canis* isolates, only two were compatible with the tester strain of *N. otae* (–), and 27 isolates were with *N. otae* (+). No gymnothecium was produced by 33 isolates of *M. canis* and the 54 isolates of *M. equinum* in combination with tester strains.

Introduction

Microsporum canis is the main causative agent of canine and feline dermatophytosis throughout the world. The perfect state of *M. canis* was confirmed to be *Nannizzia otae* by Hasegawa & Usui in 1975 (4). This species is heterothallic and composed of either (+) or (–) mating types. Recently, in a study of the mating behaviour of *N. otae,* most of *M. canis* were found compatible with *N. otae* (+) or to be non-reactive isolates (5, 7, 15). *M. equinum,* described as synnonym of *M. canis* before, has been present in Hokkaido of northern Japan in 1972 (12). Padhye, Weitzman & Ajello made a study of the mating behaviour of *N. otae* with *M. equinum* isolated from 12 countries (10). However, no gymnothecium from crosses between *M. equinum* and *N. otae* was produced.

During the course of our study on the causative agents of dermatophytosis, *M. canis* and *M. equinum* was isolated from animals. A determination of their mating types was carried out.

This paper presents the results of an investigation on the mating types of heterothallic species and a description of a canine and feline isolates of *N. otae* (+).

Materials and methods

One hundred and ten isolates of *M. canis* and *M. equinum* in Japan were collected from various types of animals over a period of 5 years from 1976 to 1981. These dermatophytes were isolates from animal skin lesions. The areas from which the animals were obtained are appear in Figure 1. Fifty six *M. canis* isolates were obtained from 41 dogs, 14 cats and one tiger. The fifty four *M. equinum* isolates came from equine dermatophytosis in Hokkaido.

Tester strains of *N. otae* used were (+) VUT 77054 and (–) VUT 77055 which were produced by the F_1 progeny of VUT 73015 and VUT 74001, respectively.

The *M. canis* and *M. equinum* isolates were paired with tester strains of *N. otae* on Weitzman Silva-Hutner medium ($MgSO_4$ $7H_2O$ lg., KH_2PO_4 lg., $NaNO_3$ lg., Beech Nut Baby Oatmeal 10 g., agar 18 g. and water 1000 ml) adjusted to pH 5.6. The plates were incubated for 8 weeks at 22 ± 1 °C.

The mating behaviour of *N. otae* between (+) 76-OU-1 derived from dog in this paper and (–) VUT 77055 on several kinds of media was also investigated. Weitzman Silva-Hutner, YpSs, potato dextrose, corn meal and Sabouraud's dextrose agar were used as non-keratinous media. Soil hair medi-

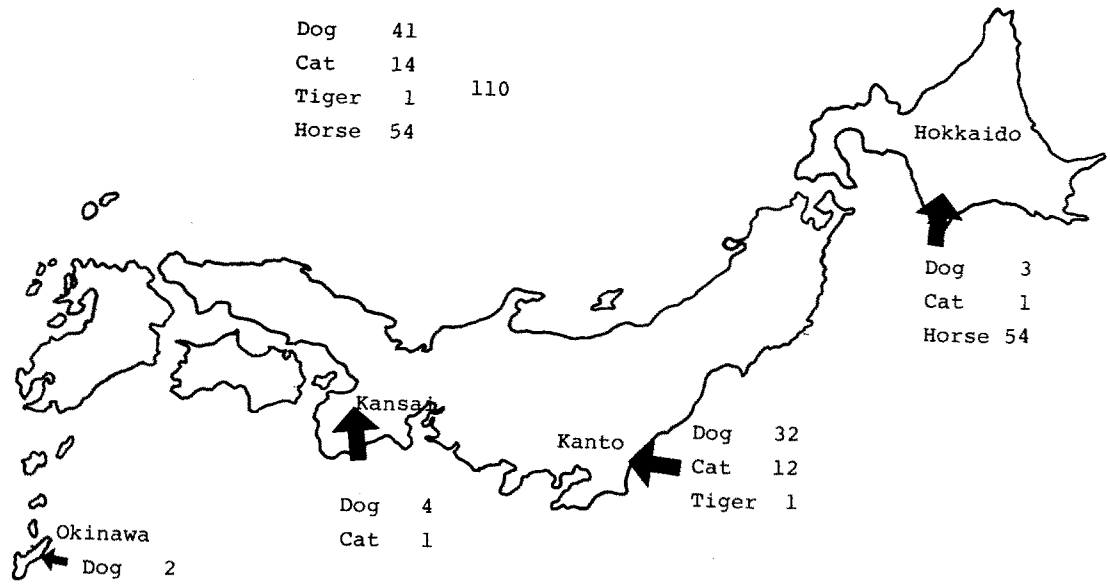


Fig. 1. Microsporum canis and *M. equinum* isolates examined for mating types.

um were from horse, human, dog, cat and guinea pig. The plates were incubated at 22 ± 1 °C and examined over a period of 8 weeks.

Results

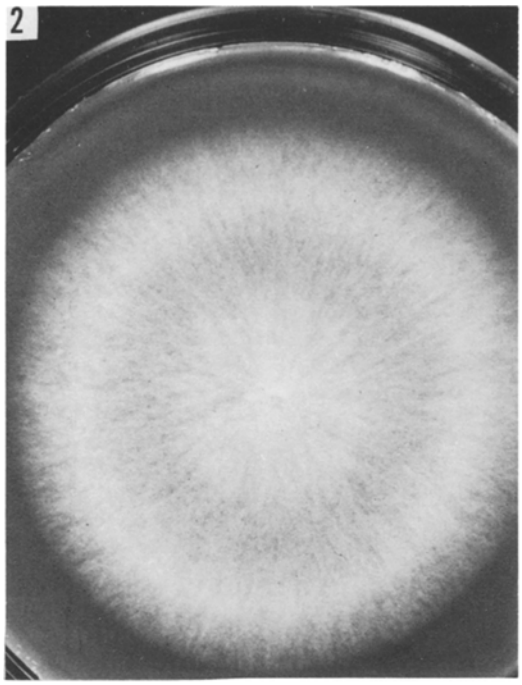
1. Mating studies

The results of mating experiments conducted on the 56 isolates of *M. canis* and 54 isolates of *M. equinum* are shown in Table 1. Of the 56 *M. canis* isolates, only two, one from a dog and one from a cat, were confirmed to be *N. otae* (+) strains. Twenty seven isolates of this species were *N. otae* (–) strains; 21 of these came from dogs, 5 from cats and one from the tiger. Of the 54 *M. equinum* isolates from horses, none produced fertile gymnothecium or pseudogymnothecium throughout the 8-week incubation period. The two isolates of (+) mating type were originated from Hokkaido of northern Japan.

Table 1. The mating reactions of *M. canis* and *M. equinum* isolates from animals.

District	Animal	Number of isolates	Isolates producing gymnothecia with		Non-reactive isolates
			(+) mating type	(–) mating type	
Hokkaido	Dog	3	1	1	1
	Cat	1	1		
	Horse*	54			54
Kanto	Dog	32		19	13
	Cat	12		4	8
	Tiger	1		1	
Kansai	Dog	4		1	3
	Cat	1		1	
Okinawa	Dog	2			2
Total		110	2	27	81

* *Microsporum equinum*.



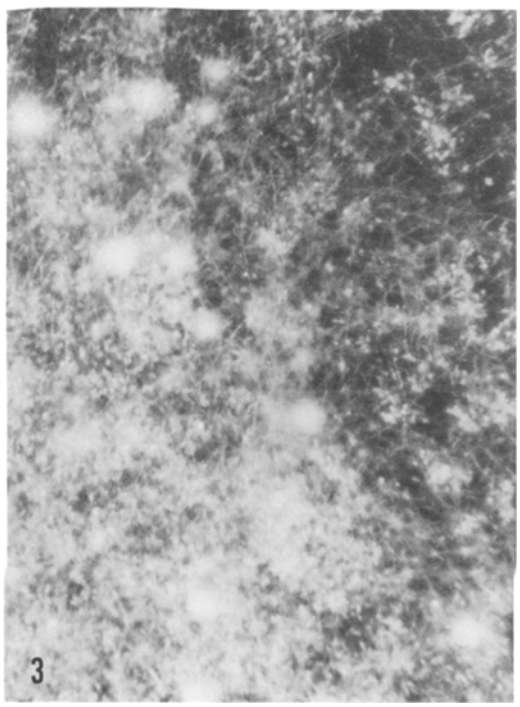


Fig. 2. Four week-colony of *N. otae* (+) 76-OU-1 on YpSs medium at 25 ° C.
Fig. 3. Gymnothecia on Weitzman Silva-Hutner medium (*N. otae* 76-OU-1 × tester *N. otae* (–) strain).
Fig. 4. Small amount of gymnothecia on human air of keratinous medium. (*N. otae* 76-OU-1 × tester *N. otae* (–) strain).
Fig. 5. Cigar shaped macroconidia around the gymnothecium.

Table 2. Sexual reproduction in *Nannizzia otae* (= *M. canis*) on several media.

Cross	Medium	Non keratinous medium					Keratinous medium (soil-hair medium)				
		W.S.H.	YpSs	PDA	CMA	SDA	Horse	Human	Dog	Cat	Guinea pig
Isolate 76-OU-1 × VUT 77055 (-)	No. gymnothecia per plate	55–134	–	–	–	–	98	17	–	–	–
	Size of gymnothecia (μm)	290–460					380–560	390–550			
	Gymnothecia producing time	3 w.					3 w.	4 w.			
VUT 77054 (+) × VUT 77055 (-)	No. gymnothecia per plate	78–204	–	–	–	–	127	49	–	–	–
	Size of gymnothecia (μm)	310–430					390–550	400–530			
	Gymnothecia producing time	3 w.					3 w.	3 w.			

2. Mating behaviour of the N. otae (+) 76-OU-1 strain

Mating behaviour was studied between *N. otae* (+) 76-OU-1 (Fig. 2) isolated from dog and tester strain VUT 77055 on several kinds of media. Five kinds of non-keratinous media and soil hair media containing the hair of 5 species were studied to determine the mating behaviour of the *N. otae* (+) 76-OU-1 strain. The results are shown in Table 2. For the non-keratinous media, mating compatibility could be confirmed only on the Weitzman Silva-Hutner medium (Fig. 3).

On the keratinous soil and hair media, mating compatibility was found in the case of both horse and human hair (Fig. 4). From 55 to 134 gymnothecia or pseudogymnothecia were produced on Weitzman Silva-Hutner medium (Fig. 5). The size of them, however, varied from 290 to 560 μm in diameter according to the primary or mature state. Crosses were also carried out between *N. otae* (+) VUT 77054 and *N. otae* (-) VUT 77055, and the results obtained with the tester strains of *N. otae* appear in the lower part of Table 2. The gymnothecia or pseudogymnothecia from both mating were probably produced from various kinds of non-keratinous and keratinous media.

Discussion

Nannizzia otae is a heterothallic ascomycete. Its mating behaviour has been studied since 1975 because of the scarcity of the (+) mating type strain. The affirmations of the *N. otae* (+) mating type were reported by Hasegawa & Usui (4) and Hironaga, Nozaki & Watanabe (5). The later workers found that among 119 *M. canis* isolates from human dermatophytosis, 112 proved to be of the (-) mating type, but only one isolate was the (+) mating type. However, some workers in Japan failed to isolate the (+) mating type (7, 8).

Weitzman & Padhye (15) reported that of 198 *M. canis* isolates collected from North America, Europe, South Africa and Oceania, 141 were the (-) mating type and none could be confirmed as the (+) mating type. In this study, among the 56 *M. canis* strain, 27 were (-) mating type and only 2 were (+) mating type isolated from cat and dog of house keeping. *M. canis* of tiger dermatophytosis formerly published was not (+) mating type but (-) mating type (13).

From a mycological standpoint, *M. canis* is considered by some mycologists to be similar to *M. equinum* (1, 2, 3). But quite recently, Padhye et al. (10), Kane et al. (6) come to the conclusion that *M. equinum* differs from *M. canis* in macroconidia size, and because of its inability to perforate hair *in*

vitro and its incompatibility with *N. otae*. The incompability between *N. otae* and *M. equinum* was found in our work as well as in that of Padhye, Weitzman & Ajello (10). Thus, *M. equinum* isolated from horses and *M. canis* are genetically two distinct species.

The country from which all the 4 *N. otae* (+) strains originated has been Japan. They were obtained from 2 cats, human and dog. Of the 4 isolates, 3 *N. otae* (+) strains derived from animals were discovered only in Hokkaido district in Japan. From this fact, it will be briefly investigated the mating type of *M. canis* isolated from animals in Hokkaido. A pomeranian dog and a Persian domesticated cat infected with the *N. otae* (+) strain were keeping in house. However, the manner in which they became infected remains unclear.

Though *N. otae* (+) mating type was demonstrated by primary mating experiment on Weitzman Silva-Hutner medium, it should be studied whether any experiments were superior to produce the gymnothecia. According to Hironaga, Nozaki & Watanabe (5), niger seed salt agar is the most suitable media for producing the gymnothecia of *N. otae*. The other workers have been studied on the ascocarp reproduction of some Nannizzia species using several kinds of keratinous or non-keratinous media (9, 11, 14). Our data that the gymnothecia were produced on non-keratinous Weitzman Silva-Hutner medium and the keratinous medium contained human and horse hair did not completely confirm that these media are the most ideal for sexual reproduction.

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