# Distribution of mating types among clinical isolates of the *Microsporum gypseum* complex

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## Abstract

Forty-five clinical isolates of *Microsporum gypseum* were crossed with the + and - tester strains of *Nannizzia gypsea, N. incurvata* and *N. fulva* to determine their mating type. Thirty-two produced fertile gymnothecia when crossed with *N. gypsea,* of which 13 reacted as + and 19 as -. Ten produced fertile gymnothecia when crossed with *N. incurvata,* of which 7 reacted as + and 3 as -. The remaining 3 were sterile in all crosses with *N. gypsea, N. incurvata* and *N. fulva.* Of these 3, 2 were fluffy, degenerated cultures of *M. gypseum,* and the other one appeared to be identical with the discription of *M. gypseum* var. *vinosum* in cultural and morphological characteristics. There was approximately a 1:1 overall ratio of mating types among a total of 90 clinical isolates of *M. gypseum* in Japan on the basis of the above results and the data recently reported in the literature by other Japanese workers. The present results suggest that the ability to cause skin infection of humans by the soil-inhabitating organisms is approximately equal in the mating types and probably independent of sexuality.

## Introduction

*Microsporum gypseum* (Bodin) Guiart & Grigoraskis is a common dermatophyte and an occasional causative agent of human and animal dermatophytoses. The perfect states of this imperfect species were discovered and reported as *Nannizzia incurvata* Stockdale (12) and *N. gypsea* (Nann.) Stockdale (13). Stockdale (13) proposed that *M. gypseum* was a complex composed of different asexual strains corresponding to the conidial states of *N. incurvata, N. gypsea,* and also of *N. fulva* Stockdale.

The present study was undertaken to ascertain the distribution of mating types among clinical isolates of the *M. gypseum* complex recovered in Japan for an epidemiological and ecological approach to this species complex.

## Materials and methods

### *Tester strains and isolates*

Tester strains of *N. gypsea* (CMI 86175+ and CMI 86176-), *N. incurvata* (CMI 104076+ and CMI 86518-) and *N. fulva* (CMI 86179+, CMI 86180-, CMI 99944+ and CMI 99943-) were provided from the Commonwealth Mycological Institute, Kew. Forty-five strains used for crosses were from the culture collection in the Department of Dermatology, Shiga University of Medical Science, Otsu, Japan (SM no. is the accession number of cultures). They were isolated from human skin lesions, mostly on exposed areas, in seven different areas of Japan (Table 1).

### *Mating experiments*

All of the 45 isolates were crossed with all of the

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tester strains of *N. gypsea, N. incurvata* and *N. fulva* on Weitzman & Silva-Hutner's medium (13) with a small amount of powdered horse-hair added on the agar plates. Isolates which were sterile when crossed on the agar medium were also paired with the tester strains on soil and hair medium in Petri dishes.

Each of the strains was transferred onto dilute Sabouraud dextrose agar (3) slants and incubated for 1 wk at 26–27 °C before being used for crosses.

*Table 1.* Clinical isolates of the *Microsporum gypseum* complex.

| Strain No. | Mating type | Patient Age Sex | Clinical lesion | Area | Year |
|---|---|---|---|---|---|
| SM 8063 | *N. gypsea* – | 7 N | kerion celsi | Shizuoka | 1976 |
| SM 8127 | *N. gypsea* + | 68 F | kerion celsi | Shizuoka | 1979 |
| SM 8210 | *N. gypsea* – | 2 F | kerion celsi | Shizuoka | 1980 |
| SM 8068 | ? | 14 M | tinea (scrotum) | Hamamatsu | 1976 |
| SM 8129 | ? | 10 F | tinea faciei | Otsu | 1979 |
| SM 8137 | *N. gypsea* + | 6 M | tinea corporis | Otsu | 1979 |
| SM 8138 | *N. gypsea* – | 1 M | kerion celsi | Otsu | 1979 |
| SM 8250 | *N. gypsea* + | 22 M | tinea (scrotum) | Otsu | 1980 |
| SM 8251 | *N. gypsea* + | 73 F | tinea manum | Otsu | 1980 |
| SM 8054 | *N. gypsea* + | 4 M | tinea (leg) | Kyoto | 1970 |
| SM 8069 | ? | 18 F | tinea (buttock) | Kyoto | 1962 |
| SM 8079 | *N. gypsea* + | 14 F | tinea manum | Kyoto | 1973 |
| SM 8095 | *N. gypsea* – | ? | tinea capitis | Osaka | 1978 |
| SM 8105 | *N. gypsea* – | 64 F | tinea faciei | Osaka | 1977 |
| SM 8070 | *N. incurvata* – | 26 F | tinea manum | Tenri | 1971 |
| SM 8080 | *N. incurvata* + | 5 F | tinea faciei | Tenri | 1971 |
| SM 7127 | *N. gypsea* – | 8 M | tinea capitis | Wakayama | 1974 |
| SM 7416 | *N. gypsea* – | 21 M | tinea (forearm) | Wakayama | 1976 |
| SM 7442 | *N. gypsea* + | 55 F | tinea (wrist) | Wakayama | 1976 |
| SM 7502 | *N. gypsea* – | 3 M | kerion celsi | Wakayama | 1976 |
| SM 7538 | *N. gypsea* – | 1 F | tinea (chest) | Wakayama | 1977 |
| SM 7557 | *N. gypsea* – | 12 F | tinea corporis | Wakayama | 1977 |
| SM 7565 | *N. gypsea* – | 1 M | tinea (groin) | Wakayama | 1977 |
| SM 7618 | *N. gypsea* + | 47 F | tinea faciei | Wakayama | 1978 |
| SM 7336 | *N. incurvata* + | 2 M | tinea faciei | Wakayama | 1976 |
| SM 7504 | *N. incurvata* – | 15 F | tinea faciei | Wakayama | 1977 |
| SM 8057 | *N. gypsea* – | 5 F | tinea corporis | Oita | 1977 |
| SM 8058 | *N. gypsea* + | 64 F | tinea corporis | Oita | 1977 |
| SM 8059 | *N. gypsea* + | 29 F | tinea manum | Oita | 1977 |
| SM 8060 | *N. gypsea* – | 37 F | tinea manum | Oita | 1977 |
| SM 8061 | *N. gypsea* – | 30 F | tinea pedis | Oita | 1977 |
| SM 8062 | *N. gypsea* – | 8 M | tinea cruris | Oita | 1977 |
| SM 8071 | *N. gypsea* – | 33 F | tinea pedis | Oita | 1977 |
| SM 8087 | *N. gypsea* – | 10 M | tinea (forearm) | Oita | 1978 |
| SM 8093 | *N. gypsea* + | 13 M | tinea cruris | Oita | 1978 |
| SM 8140 | *N. gypsea* – | 14 M | tinea corporis | Oita | 1979 |
| SM 8186 | *N. gypsea* + | 6 M | kerion celsi | Oita | 1980 |
| SM 8225 | *N. gypsea* + | 6 M | tinea corporis | Oita | 1980 |
| SM 8229 | *N. gypsea* – | 30 F | tinea corporis | Oita | 1980 |
| SM 8078 | *N. incurvata* + | 8 M | tinea faciei | Oita | 1977 |
| SM 8088 | *N. incurvata* + | 3 M | tinea faciei | Oita | 1978 |
| SM 8119 | *N. incurvata* + | 75 M | tinea (thigh) | Oita | 1979 |
| SM 8198 | *N. incurvata* + | 32 F | tinea faciei | Oita | 1980 |
| SM 8243 | *N. incurvata* – | 1 F | tinea faciei | Oita | 1980 |
| SM 8244 | *N. incurvata* + | 2 M | tinea cruris | Oita | 1980 |

Small pieces of vigorously growing culture were cut out of the colonies and inoculated approximately 1 cm apart on the center of agar plates or on soil and hair medium in Petri dishes. All tests were performed in duplicate.

The plates and Petri dishes were incubated in the dark at 26–27 °C for 2–4 wk after which they were examined for the presence or absence of gymnothecia. Whenever gymnothecia were observed, they were examined for asci and ascospores. Asci from each of the fertile crosses were dissected and ascospores were tested for germination.

## Results

*Mating experiments* (Tables 1, 3)

The 45 isolates used for crosses were all self-sterile. Of the 45, 32 produced 120–330 fertile gymnothecia per plate when paired with *N. gypsea*. Of the 32, 13 reacted as + mating type and 19 as − type. Ascospores from these fertile crosses were viable without exception when transferred onto Sabouraud dextrose agar plates and incubated at 26–27 °C.

Ten isolates produced 180–520 fertile gymnothecia per plate when crossed with *N. incurvata*. Of the 10, seven reacted as + and three as −. Ascospores from these fertile crosses were also viable.

The remaining three isolates were sterile in all crosses with *N. gypsea*, *N. incurvata*, and *N. fulva* on the agar medium and also on soil and hair medium. Of the three, two were fluffy, degenerated cultures of *M. gypseum*. The other was atypical and formed a silky colony suggestive of *M. canis* but with a reddish brown surface and undersurface. During subcultures, the organism produced several whitish and powdery sectors consisted of numerous club-shaped microaleurioconidia, 2.5–3 × 4–6 μ, and broadly-spindle to spindle-shaped, smooth-walled macroaleurioconidia, 7.5–14 × 45–60 μ, with usually up to 6 and rarely 7 septa. After careful consideration of the cultural and morphological features, we identified this atypical isolate as *M. gypseum* var. *vinosum* Gordon & Lusick (2).

*Mating types and clinical lesions* (Tables 1, 2)

Of the 32 isolates compatible with *N. gypsea*, 17 were obtained from inflammatory skin lesions on exposed areas. The other 15 isolates were from the tinea corporis, cruris or pedis in ten infants and seven adults. No exceptional difference in the number of the isolates depending on the site of the clinical lesions was observed between + and − mating types. On the other hand, out of the 10 isolates compatible with *N. incurvata*, seven were obtained from tinea faciei in five infants and two adults. Among these seven isolates, mating types were predominantly +. The remaining three were from tinea manum and tinea on the upper thigh in two adults, and from tinea cruris in a neonate.

*Table 2.* The relation between mating type and the site of clinical lesions.

| Mating type | Site of clinical lesion | | | | | |
|---|---|---|---|---|---|---|
| | Scalp[a] | Face | Trunk Groin Buttock Thigh | Forearm Wrist Leg | Hand | Foot |
| *N. gypsea* + | 2 | 1 | 5 | 2 | 2 | 1 |
| *N. gypsea* − | 6 | 1 | 7 | 2 | 1 | 2 |
| *N. incurvata* + | – | 5 | 2 | – | – | – |
| *N. incurvata* − | – | 2 | – | – | 1 | – |
| ?[b] | – | 1 | 2 | – | – | – |
| Total | 8 | 10 | 16 | 4 | 4 | 3 |

[a] Including kerion celsi.
[b] Sexually non-reacted isolates.

## Discussion

Our study revealed a 13 + : 19 - ratio of mating types among 32 clinical isolates of *N. gypsea* and a 7 + : 3 - ratio among 10 clinical isolates of *N. incurvata*. To our knowledge, in Japan, a total of 45 cases of *M. gypseum* infection in which causative agent was identified as *N. gypsea* or *N. incurvata* by mating experiments have been reported in the literature (6–8, 11, 18) (Table 3). Soh (11) reported a 17 + : 9 - ratio of mating types among their 26 clinical isolates of *N. gypsea*, and a 1 + : 4 - ratio among five clinical isolates of *N. incurvata*. Iwashige (6) and Iwashige *et al.* (7) reported a 5 + : 7 - ratio of mating types among a total of 12 clinical isolates of *N. gypsea*. In addition, Tomisawa *et al.* (18) described a case of tinea unguium caused by the + type of *N. gypsea* in an infant, and Nishimoto *et al.* (8) found a case of tinea faciei by the - type of *N. incurvata* in an infant.

Therefore the overall ratio of mating types among clinical isolates of *N. gypsea* and *N. incurvata* hitherto examined in Japan are approximately 1:1, although the ratios varied considerably within the areas (Tables 1 and 3). The results well correspond to those of Weitzman *et al.* 's work which showed approximately a 1:1 ratio of mating types among 58 American isolates from clinical materials (20).

The equal distribution of mating types among clinical isolates of the *M. gypseum* complex suggests that the ability to cause skin infection of humans by the organisms is approximately equal and probably independent of sexuality. However, further investigations on the distribution of mating types among soil isolates of the geophilic dermatophytes as well as other soil-inhabitating pathogenic fungi are necessary to prove this possibility. Recently Gaur & Lichtwardt (1) have reported a ratio of approximately 1:1 of mating types among 338 soil isolates of *Histoplasma capsulatum*, whereas most of the isolates from clinical materials are of the - mating type. We have also performed mating studies with many soil isolates of *M. gypseum* obtained from various sites in Japan and found approximately a 1:1 overall ratio of the mating types (unpublished data).

Although the *M. gypseum* complex, as representative of the geophilic dermatophytes, shows an equal distribution of mating types among its clinical isolates, most anthropophilic dermatophytes are known to occur as only + or - mating types: e.g., *Trichophyton rubrum* (Castellani) Sabouraud occurs as only - mating type, whereas *T. interdigitale* Priestley and the *'powdery'* and *'persicolor'* forms (anthropophlic variants) of *Arthroderma vanbreuseghemii* Takashio occur as only + mating types (3, 5, 14, 15, 23). Also, in some zoophilic dermatophytes, either of the mating types is predominant among their clinical isolates: e.g., in *N. otae* Hasegawa & Usui (=*M. canis* Bodin), mating types are almost entirely - (4, 21), and in the *granulosum-asteroides* form of *A. vanbreuseghemii*, mating types are predominantly + (3, 5, 15, 17). These epidemiological facts suggest that some unknown selective factors have continuously acted on the mating types of each dermatophyte during evolution to become zoophilic or anthropophilic and dissemination.

For an explanation of such unequal distribution

*Table 3.* Mating types among a total of 90 clinical isolates of the *Microsporum gypseum* complex obtained in Japan.

| Author(s) | Present study | Soh (11) | Iwashige (6), Iwashige *et al.* (7) | Tomisawa *et al.* (18) | Nishimoto *et al.* (8) | Total |
|---|---|---|---|---|---|---|
| Area | (in Table 1) | Kobe | Tokyo | Tokyo | Nagasaki | |
| Mating type | | | | | | |
| *N. gypsea* + | 13 | 17 | 5 | 1 | – | 36 |
| *N. gypsea* - | 19 | 9 | 7 | – | – | 35 |
| *N. incurvata* + | 7 | 1 | – | – | 1 | 9 |
| *N. incurvata* - | 3 | 4 | – | – | – | 7 |
| ? | 3 | – | – | – | – | 3 |

? = sexually non-reacted isolates.

of mating types, Rippon (9, 10) suggested biochemical-genetic determinants of virulence in some dermatophytes. Weitzman & Padhye (21) suggested three possible explanations for *N. otae,* namely, 1) selective factors such as the presence of strains associated with lethal factors, 2) pathogenicity, and 3) geographic distribution. Of these possibilities, pathogenicity appears less promissing in the dermatophytes (19). However, geographic distribution is likely. In this connection, the varying epidemiological factors that continuously acted on the dermatophytes might have played an important role to selective the mating types during their dissemination dependent on animals or man.

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