Form and function in the evolution of dermatophytes

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Summary

The phenotype of dermatophytes has been radically influenced by two very different evolutionary paths: that of the mostly sexual, soil-associated species, and that of the mostly asexual, non-soil-associated species. The former category, including geophiles and some zoophiles, is characterized by well established conidial dimorphism and the presence of apparent anti-arthropod-grazing structures such as helical appendages, as well as by growth factor independence, functional urease enzyme, and hair perforation organs. The latter category, including some zoophiles and all anthropophiles, is characterized by loss of some or all of the above characters. Phenotypic characters which are retained in the non-soil-associated dermatophytes, and likely selected for in their niche, include the production of infectious "substrate arthroconidia" and the production of secondary metabolites, often seen as coloured compounds in culture. The presence of xanthomegnin and other secondary metabolites, which have no known function in pathogenesis, may reflect deterrence of bacterial competitors in skin and nails. The diversity of such compounds may reflect "anomalizing selection" operational in the evolutionary maintenance of a general inhibition of unspecialized, opportunistic competitors. These competitors are non-specifically deterred by the saturation of the fungally colonized area with large quantities of metabolically inaccessible material, which must be maintained as inaccessible by evolutionarily accelerated but relatively undirected change.

Key words

Dermatophytes, *Trichophyton*, Ecology, Evolution, Anomalizing selection

Dermatophytes and their congeneric, nonpathogenic relatives the dermatophytoids [1] are anamorphs currently classed as *Trichophyton*, *Microsporum* and *Epidermophyton*, corresponding in many cases to teleomorphs in the genus *Arthroderma*. Along with a few *Chryosporium* and *Myceliophthora* species with teleomorphs in *Arthroderma*, they compose a diverse spectrum of anamorphic fungal types associated with the family Arthrodermataceae. These anamorphs have a complex range of phenotypic presentations *in vitro*, ranging from species with dimorphic conidia to types that are completely nonsporulating. (Note that the term "dimorphic" is used in mycology in the general sense meaning "possessing two forms," and does not imply a mold-to-yeast conversion). They also include species displaying a great variety of colony pigmentation characteristics, including deep vinaceous, cherry red, lemon yellow, blue, and umber. On the other hand, the morphology of their teleomorphs is so uniform that most of the species are not morphologically distinguishable unless the anamorph is studied. The objective of this article is to review the form and function of dermatophyte phenotypes, and, as far as possible, to place the phenomena seen *in vivo* and *in vitro* into their known or likely ecological and evolutionary contexts. Discussion is limited to characters that are visible in laboratory macroscopic and microscopic examinations, as well as in common tests employing special diagnostic media. This is partly for conciseness, and partly because a distinctive picture emerges from this examination without need to resort to additional considerations.

Ecological and evolutionary overview of dermatophytes and dermatophytoids

It has long been thought, and has been confirmed by modern phylogenetic studies [2-5] that pathogenic dermatophytes probably arose from soil-borne, nonpathogenic ancestors likely similar in habitat to today's nonpathogenic dermatophytoids (e.g., *Trichophyton ajelloi*, *Trichophyton terrestre*). These fungi make up a lineage of keratinophilic organisms derived from common ancestors of the ecologically important order Onygenales, one of the few groups of fungi in which most or all members are specialized for degradation of proteinaceous substrates. Keratin is a refractory protein polymer only produced by animals, and is the main constituent of epidermal skin, hairs, feathers, reptilian scales, quills, horns, hooves, and nails. These materials, shed into or onto the soil, or onto other potentially moist substrata such as disused bird nests, are principally degraded by keratinophilic fungi and streptomycetes. Some Onygenalean fungi with macroscopic fruiting bodies such as members of the type

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genus *Onygena* may decay large keratinous substrata such as hooves. However, the minute ascomata of *Arthroderma* species, including teleomorphs of soil-associated dermatophytes, dermatophytoids, and a few closely related *Chrysosporium* and *Myceliophthora* species, are more commonly encountered on smaller substrata such as hairs, quills and feathers [6]. They are also extremely common in association with hair-filled carnivore scats [pers. obs.; Malloch, pers comm.].

Trichophyton, *Microsporum* and *Epidermophyton* species have classically been divided ecologically into soil-dwelling geophilic species, animal-associated zoophilic species, and human-associated anthropophilic species. The geophiles include both dermatophytes, i.e., species at least occasionally causing skin disease (definition as per Ajello [7]) and dermatophytoids, species never rigorously confirmed as causing disease. These species can be correctly referred to as "soil fungi" (e.g. see Domsch et al. [6]). Users of this much abused term, however, should be aware that modern soil mycology distinguishes between species which may be isolated from soil as dormant propagules alone — that is, species which have growth habitats outside the soil but which have propagules capable of surviving in soil — and species which actually grow in or on soil. Those that grow in soil do not generally grow on nonspecific substrata in a generalized soil matrix, but rather are mostly associated with particular types of substrata. In the case of filamentous fungi, these substrata are often largely or entirely macromolecular in nature: the filamentous habit of growth is a specialty for penetrating solid or semisolid matrices, but its diffuse nature tends to make it poorly competitive against closely packing particulate organisms such as bacteria and yeasts in the assimilation of small molecules in solution. Typically, soil filamentous fungi are studied by washing soil particles to remove sedimented dormant propagules, and then observing the fungi which grow as colonizers of particular types of particles [8]. Dermatophytes and dermatophytoids are specific keratinophiles, and as such are not normally isolated from humic soil aggregates, plant root materials, or other organic soil constituents (e.g., Söderström and Bååth [9]). They are instead obtained by the Vanbreuseghem technique of baiting soil with keratin, a process that presumably stimulates dormant soilborne propagules in fungistasis to begin active growth [10]. When this keratin-specificity is understood, it is easy to see that the ecological distinction between geophilic and most zoophilic dermatophytes is very subtle. Although there has been very little direct study of geophilic or zoophilic dermatophytes in their natural habitats (except basic baiting isolation studies), there is one dermatophyte structure, which by its nature cannot reasonably be predicted to form on a living animal host, and must therefore form on soil-borne material. This structure is the ascoma, the fruiting structure of the teleomorph. Although small, the ascoma is certainly large enough to be removed by scratching or rubbing. To say that it does not form on the host is not mere speculation, since in over a hundred years of dermatophyte studies no ascoma has ever been found or produced on a living animal. (The cottony gymnothecia are large enough to be readily visible to the naked eye.) Furthermore, the teleomorph does not occur at all in the dermatophytes specifically associated with species like ungulates, horses and humans which do not live in hair-lined burrows, dens or nests in association with soil (Summerbell, in Tanaka [11]). It is clear that development of teleomorphs in nature indicates a high degree of association with hair or other keratinous substrata removed from animal hosts and associated with the soil. Such an association is the necessary precondition for successful sexual reproduction.

What distinguishes teleomorph-producing zoophilic dermatophytes from their geophilic relatives is neither their degree of soil association, nor, as far as has been determined, their relative ability to cause infection. Instead, the distinction lies in the adaptation of the zoophiles to grow in association with particular animal hosts. For example, *Arthroderma otae* grows in association with canines and felines, and *Arthroderma benhamiae* in association with rodents, lagomorphs and hedgehogs. Although geophilic, zoophilic and anthropophilic dermatophytes all retain some ability to infect diverse species, including species that are not usual hosts, ongoing colonization of populations has never been shown for hosts other than the well-characterized normal hosts. For example, *Trichophyton mentagrophytes* is not uncommon from infected horses or cats, but to our knowledge there has not been any published description of a case of an extended contagious transmission or endemic establishment of *T. mentagrophytes* in horse or cat populations (as opposed to a short-term outbreak based on limited animal-to-animal transmission, or based on acquisition of infection from a common environmental source). On the other hand, horses are perennial carriers of their particular zoophile *Trichophyton equinum*, while the tenacious establishment of *A. otae* (commonly reported as the anamorph *Microsporum canis*) in catteries is well known to breeders and veterinarians (e.g., Moriello et al. [12]). In human outbreaks, which are easier to study than animal outbreaks, it can be seen that non-host-adapted species like *M. canis* may cause some sequential human-to-human transmission [13, 14], but the virulence of inoculum usually does not endure beyond a small number of transmission events. Geophilic dermatophytes such as members of the *Microsporum gypseum* complex may have a similar limitation of long-term transmission in all animal species; if they were able to maintain constant populations in association with particular animals, and regularly cause infection, they would have to be reclassified as zoophiles. Such associations cannot be absolutely excluded, but have not been reported for this group of geophiles or others. On the other hand, if their virulence is in any way maintained by selection (that is, if it requires regular or periodic animal infection to be maintained in the population), rather than being purely an accident of their keratinophily, they will be truly intermediate between geophiles and zoophiles.

Although the epidemiological distinction between geophiles, zoophiles and anthropophiles holds true, dermatophyte morphology and physiology relate more profoundly to another categorization. Dermatophytes may be classified as soil-associated or non-soil associated [1,11] (Table 1). The category of soil-associated dermatophytes includes some zoophiles and all pathogenic geophiles. These species possess sexual reproduction, copious and dimorphic conidiation, ability to perforate shed hair with perforating organs (as seen in the *in vitro* hair perforation test), vitamin independence, urease activity, and other characteristic features (Table 2) that will be discussed in more detail below. Typical examples are the *M. gypseum* complex, *M. canis*, and zoophilic members of the *T. mentagrophytes* ss. lat. complex. Non-soil-associated dermatophytes strongly tend to have lost sexual reproduction and usually have conspicuously attenuated conidiation, with macroconidia, microconidia or both becoming uncommon or rare. Stimulation with mating type strains of *Arthroderma simii* in the Stockdale test often shows that all available strains of these species react as members

Table 1. Ecological, phenotypic and population genetics classification of dermatophytes according to presence or absence of a soil-adapted, potentially sexual stage in the life cycle.

Soil-associated, sexual dermatophytes	Non-soil-associated, asexual dermatophytes	Status uncertain
Geophilic[1]	Zoophilic	*Microsporum gallinae*
		M. praecox
Microsporum boullardii	*Microsporum equinum*	*Trichophyton longifusum*
Microsporum fulvum		
M. gypseum	*Trichophyton equinum*	
M. racemosum	*T. sarkisovii*	
M. vanbreuseghemii	*T. verrucosum*	
Trichophyton vanbreuseghemii		
Zoophilic	Anthropophilic	
Microsporum canis	*Epidermophyton floccosum*	
M. persicolor	*Microsporum audouinii*	
M. nanum	*M. ferrugineum*	
Trichophyton mentagrophytes	*Trichophyton concentricum*	
(pro parte)	*T. gourvilii*	
T. simii	*T. kanei*	
	T. krajdenii	
	T. megninii	
	T. mentagrophytes (pro parte)	
	T. raubitschekii	
	T. rubrum	
	T. schoenleinii	
	T. soudanense	
	T. tonsurans	
	T. violaceum	
	T. yaoundei	

[1]Note: Geophilic dermatophytoids such as *Trichophyton terrestre*, *Microsporum cookei* and *Trichophyton ajelloi* are not included in this table, which pertains only to species normally with pathogenic potential.

of a single mating type [15]. The other mating type may have been lost over time, or may be extremely rare; or, alternatively, the entire species may be clonally derived from one or a small group of strains all belonging to the same original mating type. These non-soil species usually have also lost the ability to elaborate hair perforating organs [16], or the ability to degrade urea, or the ability to produce one or more vitamins or growth factors, or a combination of the foregoing. Typical examples are the cattle ringworm fungus *Trichophyton verrucosum*, the horse dermatophyte *T. equinum*, and anthropophiles such as *Microsporum audouinii*, *Trichophyton soudanense*, and *Trichophyton rubrum*. There is a small amount of intergradation between these categories, mainly found in anthropophilic *T. mentagrophytes* (*T. interdigitale* ss. lat.) isolates that, while seldom sexual, and mostly of a single mating type [17] have retained hair perforation, vitamin autotrophy and urease.

In examining recent phylogenetic dendrograms [2-5] showing the evolutionary relationships of the dermatophytes, it is evident that non-soil-associated dermatophytes have arisen independently from several different lineages of soil-associated species. For example, the asexual anthropophiles *M. audouinii* and *M. ferrugineum* have arisen from the *M. canis* lineage, *Trichophyton tonsurans* and *T. equinum* derive from the *Arthroderma vanbreuseghemii* line, *T. schoenleinii* derives from the *A. benhamiae* line, and more specifically, from the infraspecific line which also engendered *T. mentagrophytes* var. *quinckeanum*, the mouse favus fungus. Most non-soil-associated dermatophytes, whether zoophilic or anthropophilic, are on small, separate offshoots from soil-associated zoophilic lines. There is one exception, a major offshoot from within the *T. mentagrophytes* complex that engendered a series of anthropophiles, namely, the African endothrix *tinea capitis* fungi (*T. soudanense* and *T. violaceum*), *Trichophyton megninii*, and the *T. rubrum* complex. In each of the separate lineages where non-soil-associated dermatophytes arose, most recognizable taxa lost two or more of the "soil association characters" mentioned above: sexuality, heavy dimorphic conidiation, hair perfo-

ration, autotrophy and urease. Their animal associations were characteristically with species not dwelling in hair-lined earthen holes, such as humans, ungulates and equines, and the loss of soil association characters can be seen as the loss of characters which were no longer advantageous to organisms specialized for association with strictly epigeous animals. Although the association between ascoma formation and soilborne substrata is self-evident, as mentioned above, the connection of some of the other characters found in soil-associated dermatophytes to the soil habitat is not immediately clear. Analysis of existing data about these species, however, as well as comparison with parallel phenomena in other groups of fungi, may shed some light on the function and derivation of these characters. Individual characters will be discussed below. Although the main emphasis will be on functional morphology, physiological characters will be discussed where this sheds essential light on the ecological relationships driving the morphological evolution of these fungi.

A cautionary note concerning the attribution of functionality to biological characters

In the absence of substantial evidence about the functionality of most dermatophyte characters, this article will be directed toward pointing out likely possibilities, mostly based on analogies with evidence obtained for similar features in other groups of fungi. Some hypotheses will be generated in the hopes that they may stimulate and direct some experimentation and observation in this languishing field of dermatophyte research.

In any analysis of the functionality of biological characters, however, some precautions need to be taken. Firstly, there are many examples in evolution where intimately similar functionality has been acquired by two different types of structures. A classic example is the bird wing vs. the bat wing. Neodarwinian evolutionary philosophies, stressing the gradual evolutionary impact of

Table 2. Characters indicating ecological status of dermatophytes and dermatophytoids with regard to their relationship with soil habitats.

Soil association characters in dermatophytes and dermatophytoids	Characters suggesting dissociation from soil habitat in dermatophytes[1]
Sexual reproduction	Clonality; single mating type in *Arthroderma simii* challenge
Conidial dimorphism	One conidial type or conidia in general rare or not found in aerial mycelium
Arthropod antipredation devices: helical appendages; roughened, curved, densely rebranched structures with ossiform cells; macroconidia may be encrusted and/or rostrate and/or heavily walled	No or attenuated anti-arthropod structures: scattered, sometimes poorly formed helical appendages and inconsistently encrusted, beaked or thick-walled macroconidia vestigial in some species
Hair perforation organs	No hair perforation organs
Independence of exogenous growth factors (vitamins, amino acids)	Exogenous growth factor requirements
Urease activity	No or weak urease activity
Osmotolerance	Intolerance of concentrated media (growth strongly restricted on 5% sodium chloride Sabouraud agar)
Constitutive proteolysis	Proteolysis repressible by small molecules

[1]Non-soil-associated dermatophytes will usually show two or more, but not necessarily all, of these characters.

minor character changes, have sometimes impressed biologists as implying that every aspect of every character must have a unique adaptive significance. On the other hand, taxonomists have traditionally looked for and emphasized the seemingly arbitrary differences between structures of equivalent function (e.g., number of parts in the plant flower, type of centrum in developing ascomata) as the most reliable phenotypic indicators of phylogenetic relationship. The modern search for dissimilar sequences in equivalently functional molecules such as the ribosome is merely a cladistically compatible extension of this Linnaean point of view. The present author affirms the probable correctness of the contention that two somewhat different structures may have functions so strongly overlapping that the adaptive differences between them are rendered minor, or "neutral" [18]. Hence there will be no attempt in this essay to speculate about unique functions that may distinguish pairs of characters which are more likely to be functionally interchangeable (or, to use the most succinct English word, "fungible"). For example, the author would not contend that the nodulose cells composing peridial hairs of *Arthroderma gypsea* are in some way functionally different from the ossiform cells seen in *A. benhamiae*. If some evidence were to emerge for a functional difference between these types of cells, it would of course be taken seriously; but, in the absence of such evidence, no spurious difference will be hypothesized.

Secondly, it should be kept in mind that adaptive characters within an organism may primarily have an internally coordinating, signalling, or constructive organizational function (genes for proteins with such functions have become known as "housekeeping genes"), and may be very limited in interactions with external factors such as competing organisms or physical exigencies. There will therefore be no attempt in this writing to hypothesize external interactions to account for characters, which may not be selected for by such interactions at all. For example, the morphology of ascogonial and antheridial sexual structures is unlikely to be strongly selected by external forces, apart from general physical size and surface area/volume constraints. It should be taken as a general rule that the more complex and structured an organism is, the more of these internally coordinating characters will involve visible morphology. Hence complex fungal teleomorphs will generally have more such characters discernible under the microscope than simple anamorphs.

Characters of the teleomorph

The teleomorphs of the dermatophytes and their *Arthroderma* relatives are simple and show remarkably little variation among species. Few if any actual experiments have been done concerning the functional aspects of teleomorphic characters. In general, however, within the Ascomycota, ascospores tend to be more resistant to adverse environmental conditions than conidia are. The formation of ascospores, then, probably implies an ecology in which resistant spores are functional; this functionality probably relates to surviving dormancy in soil or litter. Some resistant spores, however, such as those of most dung fungi, are ecologically important because they survive gut passage in herbivores and then germinate in dung deposits. It is possible that Arthrodermataceous ascospores formed on hair within rodent burrows are deposited as burrow dust on the fur of the living animals. When the animals are eaten by predators, the ascospores may survive gut passage, thus accounting for the common presence of such fungi in hair-laden dung of rodent-eating carnivores such as foxes. The alternative is that dormant Arthrodermataceous ascospores or conidia may be so omnipresent in soil surface layers that any place a hair-filled carnivore scat falls is likely to contain inoculum of specialized keratinophiles.

The life cycle of non-soil dermatophytes does not contain a sexual phase, as outlined above. Other structures, especially arthroconidia formed in host skin and then shed as fomites, may function as dormant environmental propagules. Such fungi, however, have not been recorded from active growth on carnivore dung or other such environmental materials where the occurrence of geophile ascomata may be common. It is likely that all sexual dermatophytes are able to amplify their inoculum levels in the soil environment, while asexual dermatophytes may persist on some environmental materials (e.g., *T. verrucosum* fomites on cattle yard fenceposts) but have never yet been shown to amplify or reproduce there. The amplification of these fungi may be restricted to growth on animal hosts.

Examination of arthrodermataceous ascomata reveals a major functional theme in dermatophyte/dermatophytoid structures, namely, resistance to arthropod grazing. The sexual fruiting bodies are gymnothecia, a term describing the surrounding of the fertile developing centrum with a bushy open network of branches. The overall structure is a small, round network of tough branches,

somewhat suggestive of a miniature tumbleweed (also known as Russian thistle, *Salsola pestifer* Nels.). On a finer scale, the branches themselves are composed of a rebranched, curving series of nodulose or ossiform cells, that is, cells with periodic swellings or with swellings at the proximal and distal ends. These cells are conspicuously roughened. Branches terminate in long helical appendages (also called "spirals" or "spiral appendages") with sharply pointed ends. The structure as a whole is a formidable protective unit. The present author in 1989 experienced a major laboratory outbreak of fungivorous mites in stored cultures associated with mating experiments involving *A. benhamiae* and *A. vanbreuseghemii*. Conidial and filamentous material on the invaded 1/10 Sabouraud agar cultures was eaten entirely, including pseudogymnothecial clumps containing masses of conidia and diffusely distributed helical appendages, but mature ascomata were left intact. These structures, then, are inaccessible to at least some important grazers. Since the process of making specialized sexual spores may require more time than the making of conidia (this is certainly the case in culture), and since the meeting of compatible mating types may itself be occasional in nature, leading to an enhanced value for protection of the ensuing progeny, there is clearly a potential for ecological advantage in differentiating arthropod-deterring structures around developing ascospores. The whole structure of ascomata, then, as examined by a fungal ecologist, is evocative of the competitive soil environment and further links species forming these structures to the soil.

It is interesting to compare the helical appendages of *Arthroderma* spp. to the much more heavily coiled helices of *Ajellomyces* species and the comb-like appendages of the closely related [19] *Ctenomyces*. These seem to be likely examples of fungible characters, i.e., one form is probably approximately as functional as another in meeting the same environmental exigency of deterring non-specific grazers. Experimentation could readily clarify the degree to which each type of structure is efficacious against particular grazing arthropod species.

The very constancy of ascomatal structure in the Arthrodermataceae raises another matter of interest. Recent phylogenetic studies have shown an apparent association between sexuality in dermatophytes and the retention of plesiomorphous characters. For example, the three major clades of the *T. mentagrophytes* complex, the *A. vanbreuseghemii* clade, the *A. benhamiae* clade and the *A. simii* clade, are morphologically indistinguishable or nearly so both in their anamorphs and in their teleomorphs, particularly in strains which retain mating competence. The asexual clades which they have given rise to, however, have become highly differentiated in micromorphology, physiology (e.g., vitamin requirements, growth rate) and pigmentation. In the sequences of phylogenetically interesting genes such as the ribosomal spacers, there is as much or more change within the phenotypically plesiomorphous *T. mentagrophytes* complex isolates as there is between these isolates and the phenotypically apomorphous asexual species their immediate common ancestors have given rise to. There has been much debate in recent decades about the extent to which sexuality functions as a stabilizing force in population genetics. While the present author does not wish to comment here on this general question, an apparent association between sexuality and morphological conservatism is evident throughout the Arthrodermataceae. It reaches its extreme in the homogeneity of teleomorphs within the entire group. Only the very minor and likely fungible character of nodulose vs. ossiform cells in gymnothecial branches is of use in distinguishing teleomorphs. Gymnothecia observed on natural substrata (e.g., *Arthroderma quadrifidum* sent to the current author on shed porcupine quills) must be cultured to determine their species identification.

Characters of the anamorph

Conidia of the aerial mycelium

Dermatophytes form three types of conidia in aerial mycelium in pure culture. All are fundamentally similar in their release by lytic dehiscence of an empty disjunctor or separating-cell, and all intergrade in at least some species. Their dehiscence mechanism may be fungible with various others found in hyphomycetous fungi, and seems to be an indicator of affinity to the order Onygenales, where anamorphs generally have this mechanism, rather than a specific adaptation. The three types of conidia are macroaleurioconidia (vernacular "macroconidia"), formed from strongly expanded, determinate, pluricellular side branches, microaleurioconidia (vernacular "microconidia"), formed from slightly expanded, unicellular side branches, and aerial arthroconidia, formed from the breakup of aerial mycelium into typical Onygenalean alternate arthroconidia [20, 21], better known from *Coccidioides immitis*. Macroconidia may intergrade with microconidia, particularly in the *T. terrestre* complex, and they may break up into arthroconidia, as in *Trichophyton vanbreuseghemii* [22], *T. kanei* [21], and "proliferans-like" variants of anthropophilic *T. mentagrophytes* [23]. The degree of plasticity seen renders all the more interesting the fundamental question concerning how such a diversity of conidiation may be stably maintained by natural selection.

The number of hyphomycetous fungi showing unicellular vs. multicellular dimorphism in their germinable conidia is relatively small. Apart from *Trichophyton* and *Microsporum*, well-known examples include the closely interrelated *Fusarium* and *Cylindrocarpon*. In no case is it clear what selective forces have engendered the conidial diversity. Since many Onygenalean fungi, including some *Arthroderma* anamorphs, have conidia dimensionally equivalent to microconidia (whether as aleurioconidia or arthroconidia), the conidial form that stands out as anomalous is the macroconidial form.

In general, large multicellular fungal conidia are usually associated with one or more of a short list of specialized functions. Because of the large investment of energy required to produce them, they are unlikely to exist without functioning in a specialized, advantageous role. Their size may be advantageous in impaction through an aerial or aqueous boundary layer, as in *Alternaria*, *Drechslera*, and other colonizers of standing plant surfaces. Some conidia may be specialized to hook into or entangle suitable substrate surfaces, as in *Varicosporium* and other Ingoldian hyphomycetes. Large conidia may also contain a substantial food reserve, which may be advantageous in invading refractory substrates where macromolecules are available, but few small molecules are present to enable post-germination buildup of inoculum potential from smaller conidia. Finally, large conidia may occasionally serve as food baits to attract grazers, which then disperse either uneaten large conidia or associated smaller conidia.

In the dermatophytes, macroconidia of many species have structures of types often associated in fungi with deterrence of grazers. *Microsporum* macroconidia, for example, have crystal-like granular encrustation. Although to my knowledge the chemical composition of

these structures has not been studied, nor has their impact on grazing, they do strongly resemble anti-grazing encrustations produced by other fungi. *M. canis* in particular also has a beak-like, extended, pointed apex on its conidia, parallel with the extended apices of species such as *Fusarium equiseti*, and this also appears likely to be an anti-grazing device. Finally, the macroconidia of numerous soil-associated *Trichophyton* and *Microsporum* species, including *T. ajelloi*, *M. canis*, *M. cookei*, and *M. vanbreuseghemii*, have strongly thickened cells walls that very probably serve to make them less digestible and less palatable. It is unlikely, therefore, that dermatophyte and dermatophytoid macroconidia are food baits.

The most likely of the large-conidial functions mentioned above to apply in the case of dermatophytes is that of the energy reservoir. It may be a significant advantage in the invasion of hair, feathers, etc., shed onto soil or within burrows without accompanying excretions of nutritionally rich material (e.g., dung, urine) to be able to provide endogenous energy reserves sufficient for the invasion of the refractory keratin. There are many keratinophiles, e.g., the *Chrysosporium* state of *Arthroderma cuniculi*, which remain competitive without producing macroconidia. These fungi, however, may be somewhat less competitive on newly shed, micronutrient-poor keratinous materials.

It is also possible that the greater energy reserves of the larger conidia assist prolonged dormancy, but this is by no means clear. There are many small fungal spores and conidia which are able to remain dormant in soil and other materials for prolonged periods. The large size of macroconidia might make them likely targets for wall-piercing parasites such as fungivorous soil nematodes with piercing stylet mouthparts. Therefore, their longevity in soil is a moot point, although clearly susceptible to being investigated experimentally.

Very little is known about the natural dispersal of onygenalean fungi, except in the case of dimorphic systemic animal pathogens. In these cases, *Coccidioides* arthroconidia and *Blastomyces* and *Histoplasma* aleurioconidia appear to be strongly connected with airborne dispersal. The microconidia and aerial arthroconidia of dermatophytes are small and may also be adapted for airborne dispersal. Since at least a portion of the keratinous materials shed by animals may be widely distributed as separate hairs, feathers and so on, anywhere within the animals' walking or flying ranges, keratinophilous fungi may be at an advantage to produce some widely aerially dispersing conidia in order to maximize chances of contacting these substrata.

There are a number of widely aerially dispersed, multicelled conidia, most notably Pleosporalean anamorphs such as *Alternaria* and *Drechslera*. Although such conidia may be very functional in airborne dissemination once they have entered moving air masses, all aerially distributed propagules need to escape from a nearly motionless boundary layer of frictively impeded air near surfaces into the adjacent level of potentially turbulent air. The approximate height range of typical aerial boundary layers near the soil or litter-layer surface is probably well illustrated by the heights of various *Aspergillus* conidiophores, which are constructed in such a way that a stalk is extended up through the boundary layer and is then expanded to form a broad conidiogenous platform, the vesicle, in or near the turbulent zone. In the case of Pleosporalean anamorphs, conidia are probably mainly formed on aerial parts of infected plant hosts, or on recently dead plant material, and the motion of these materials in turbulent air, plus their elevation, is likely to liberate the large fun-
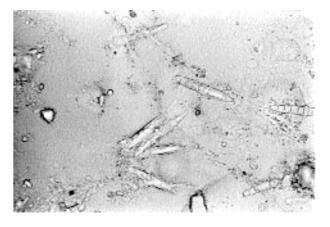

Figure 1 Dermatophytoid macroconidia strongly suggestive of *Trichophyton ajelloi* in sticky tape preparation of dust and mold near wall baseboard of chronically flooded basement (400x).

gal conidia into turbulent air well away from the ground surface. This combination of circumstances must favour airborne dispersal of relatively large phragmoconidia and dictyoconidia. Keratinous substrates, however, are much more likely to be on the surface of the ground or interleaved within upper litter layers, if not actually within the soil in sites such as rodent burrows. It seems relatively unlikely that macroconidia commonly enter the airstream from such substrata, where they would mostly be produced well within the static boundary layers of air. There is considerable "negative evidence" for the rarity of arthrodermataceous macroconidia in air, in that the many spore trapping studies performed in aerobiological research do not report the presence of these structures, which are highly recognizable in direct microscopic examination of natural materials. For example, Figure 1 shows macroconidia clearly resembling those of *T. ajelloi* in a sticky tape mount of basement wall baseboard dust in a mold-contaminated house.

It seems most reasonable to suggest, therefore, that macroconidia may be relatively strongly associated with short range dispersal. Certainly, within animal dwellings, where the animals themselves would disperse the conidia through their movements, such conidia might be highly efficacious. They would be adequately dispersed within an area where keratinous materials were concentrated, and would carry the extra energy reserves to effect rapid invasion. A physiological character, the high osmotolerance of geophilic and sexual zoophilic dermatophytes (i.e., soil-associated dermatophytes), as exemplified by their high salt tolerance [24] corroborates this scenario. The osmotolerance observed *in vitro* indicates that soil-associated dermatophytes and dermatophytoids are capable of invading very osmotically dry substrata. The only likely contenders for such substrata in their natural environments are recently shed keratinous materials, which most likely still retain parts of their original coating of hydrophobic oils and waxes when they are deposited onto or within soil or litter. The morphological and physiological characters seen are consistent with an ability to invade refractory, hydrophobic materials efficaciously. Another strongly associated feature, the ability to produce perforating organs in dissociated hair, is also consistently associated with all the soil-dwelling dermatophytes and dermatophytoids.

The production of both large and small conidia, then, may be at least partially selected for by the ongoing need to produce highly competitive conidia for short range dispersal within keratin-rich microenvironments,

and the need to produce small, dissociable conidia to reach relatively distant, newly available substrates. Related fungi not producing macroconidia, such as keratinophilic *Chrysosporium* spp., may remain competitive via greater specialization for widespread distribution — hence their inevitable predominance in studies where keratinophilic fungi in soils are studied with Vanbreuseghem's hair baiting technique (e.g. see Ramesh and Hilda, [25]). Examination of all available evidence suggests the testable proposition that not just soil-associated dermatophytes, but also nonpathogenic, geophilic dermatophytoids, may be more closely associated with sites regularly modified by animal inputs than are the purely microconidial arthrodermataceous fungi. Unfortunately, however, there has been very little investigation of the keratinophilic fungi found in actual dwellings of soil-associated wild animals.

It is possible that macroconidia combine an energy reservoir function with a grazing resistance function. Examination of mite faecal pellets (the present author often examines these pellets in indoor mold tape impressions), reveals that many fungal conidial walls pass more or less unchanged in shape through the mite digestive tract. Mite faeces from fungally colonized areas often contain large numbers of similar, relatively small fungal conidia. While there is a great variety of arthropod grazers and each one may have a different diet, it appears likely that unusually large conidia, especially if pointed, heavy-walled, encrusted, etc., may be more likely to cause difficulty in ingestion, gut passage, enzymatic and mechanical penetration, or all the foregoing, and may therefore tend to be avoided. Since keratin depositions associated with animal habitations are likely to much more stable than dispersed keratin deposits arising from random shedding onto soil, very competitive microenvironments may develop where fungus-grazing arthropods may be favoured. In such microenvironments, there would be an unusually high value for a keratinophilic fungus to produce a grazing-resistant conidial form. Hence, the ability to produce large conidia resistant to at least some grazers may be strongly favoured under these special environmental conditions. The evolution of macroconidia may in various ways (large energy reserve, grazing resistance) reflect selection in relatively stable, intensely competitive keratin depositions.

Some asexual, non-soil-associated dermatophytes such as *Epidermophyton floccosum* produce copious macroconidia in culture, but have low osmotolerance, no ability to form perforating organs, and other characters inconsistent with competitiveness in the soil environment. This seemingly anomalous matter is discussed below in connection with substrate arthroconidia.

Non-soil-associated dermatophytes in most cases have lost the ability to produce, or at least to regularly produce, one or both types of aleurioconidia. A substantial number of anthropophiles, such as *Trichophyton concentricum* and *T. schoenleinii*, never or seldom produce aerial conidia of any kind. The attenuation of the abundance and diversity of conidial production is readily seen in all asexual zoophiles and anthropophiles. For example, only a very small minority of the common *T. rubrum* isolates produce macroconidia, and, while some isolates produce copious microconidia (also sometimes abundant aerial arthroconidia), many isolates even produce few or no microconidia. The same is true of the cattle ringworm fungus, *T. verrucosum*. The effective propagation of most anthropophilic fungi, as Aljabre et al. [26] and others have elegantly demonstrated with anthropophilic *T. mentagrophytes*, is essentially entirely accomplished by substra-

Figure 2. Helically twisting macroconidia formed among helically twisting hyphae of aberrant *Trichophyton rubrum* isolate (1000x).

te arthroconidia produced within infected epidermal materials. Aerial conidia, therefore, are probably purely vestigial structures in many if not all of these fungi. They lack both a likely site of formation in nature, and the infective competence to be proposed as functional elements of the life cycle. The present author often tells students that when they see a *T. rubrum* making microconidia in artificial culture, this may well be the first time within hundreds if not tens of thousands of years that that any member of that exact clonal lineage has produced these structures. Similar cultural atavism is also seen when *T. rubrum* isolates challenged with *A. simii* testers in the Stockdale test produce sterile gymnothecia: the genetic means to do this persists, even though the single-mating-type lineages comprising *T. rubrum* have not mated in recent evolutionary time.

Consistent with this scenario is the high amount of evident genetic drift seen in the formation of aerial conidia of asexual anthropophilic dermatophytes in culture. The present author has described *T. kanei* based on a purely macroconidial and arthroconidial fungus otherwise similar to *T. rubrum*. Isolates producing mainly reduced-diameter, filiform-capitate macroconidia, as well as extended arthroconidia, are regularly seen atypical variants of anthropophilic *T. mentagrophytes* [27], while Padhye et al., 1994 [28] have described receiving several isolates of purely macroconidial *T. tonsurans*. The present author occasionally sees such genetic exotica as *T. rubrum* isolates where all the conidia, macro- and micro-, are gently helical in form (Figure 2). These types of variants are unlikely to be selected, and instead probably represent random genetic degenerations of the conidium-producing loci. Their relatively common occurrence signals the absence of selection for functional forms of aerial conidia. The various species and strains producing few or no aerial conidia provide evidence that production of these structures is by no means a prerequisite for ecological success as an anthropophilic dermatophyte or a dermatophyte of the non-soil-dwelling hoofed animals.

The only potential evidence known to the present author for environmental production of aerial conidia by anthropophilic fungi is the organism described as *Trichophyton fischeri* Kane. This heavily microconidial organism, which also produces some macroconidia and aerial arthroconidia in culture, has been isolated on several occasions from environmental materials or body sites (e.g., sputum) not associated with dermatophytosis [29]. Recent sequence analysis has shown that it has a ribosomal internal transcribed spacer 1 and 2 sequence identical to that of *T. rubrum*. The most likely explanation for the

rare isolation of this organism is that certain so-called "granular" type *T. rubrum* isolates, which produce copious microconidia in culture, may also produce microconidia when shed into the human environment on fomites, at least under conducive conditions (e.g., dampness). Whether such microconidia could retain competence as infectious structures in nature is not known; indeed, *T. rubrum* is so specifically anthropophilic that direct investigation of pathogenicity is difficult under any circumstances, and the reliability of animal models is dubious. It should be noted that *T. fischeri* differs in culture from *T. rubrum* in its ability to produce red anthraquinone pigments on a medium with an erythritol carbon source [27]. Given the similarity in its rDNA sequence to that of *T. rubrum*, this is unlikely to indicate status as a separate species for *T. fischeri*, and instead may reflect a metabolic switch associated with transient growth on small-molecule-deficient off-host keratin. Specifically, such a switch may affect the secondary carbon assimilation pathway known as the pentose phosphate pathway, in which the assimilation of exogenous erythritol would ordinarily be integrated. This character, then, may be useful for recognizing certain instances in which growth of heavily microconidial *T. rubrum*-like fungi from seemingly uninfected body sites may indeed reflect harmless environmental contamination, as asserted by Kane [30, 31].

Although, in general, production of aerial conidia by asexual, non-soil dermatophytes may be rare in nature, it is difficult to predict what the situation may be in anthropophilic *T. mentagrophytes*, sometimes called *T. interdigitale*. This group of isolates is strongly plesiomorphous in nature, and a proportion of isolates retain such soil association characters as heavy microconidiation, anti-arthropod helical appendages, relatively high osmotolerance (although lower than in zoophilic isolates, according to Kane and Fischer [24]), hair perforation, urease, vitamin autotrophy and, occasionally, in the more morphologically plesiomorphous isolates (that is, in velvety and heavily conidial isolates but not in cottony and sparsely conidial isolates), mating competence with testers. In those isolates that mate, one mating type is highly overrepresented [17]. The anthropophilic *T. mentagrophytes* isolates are suggestive of a clade or clades that have made the transition from zoophily to anthropophily comparatively recently in evolutionary history. Their morphological diversity, as seen in examples such as the reduced-macroconidial isolates mentioned above, indicates some influence of the genetic drift that has in all likelihood divested more anciently anthropophilic lineages of their soil association characters. Some remarkably divergent types, in fact, have rDNA sequences identical to those of *A. vanbreuseghemii*, the biological species associated with most anthropophilic *T. mentagrophytes*. The most intriguing example is the slow-growing, yellow, often sparsely conidial or aconidial *Trichophyton krajdenii* (previously often referred to by the nomenclaturally invalid name *T. mentagrophytes* var. *nodulare*). It is isolated purely from human lower body infections and, in most isolates, retains no morphological soil association characters at all apart from the ability to produce hair perforation organs *in vitro*. Only a few physiological soil association characters are retained: urease, vitamin independence, and constitutive proteolysis in the presence of small molecules on milk solids glucose medium (discussed below). Despite one report to the contrary [32], it is asexual [33]. The report in which it was reported as sexual also reported homothallic selfing of some *T. mentagrophytes* isolates, including members of the "nodular variety." No later investigator has been able to replicate similar findings; homothallism in the *T. mentagrophytes* complex is otherwise unknown. It is possible that some of the mating type testers used in that study were mixed or became contaminated with the opposite mating type, an occurrence which would mimic homothallism as well as producing false positive matings in challenges. The attenuation of functional soil association characters in anthropophilic *A. vanbreuseghemii* lines has evidently been more thorough in some lines than in others. There is, in fact, no reason why zoophilic *T. mentagrophytes* should not continue to produce new isolates adapted to human pathogenesis from time to time, as humans increasingly proliferate and invade the habitats of all terrestrial animals. The word "adapted" as used here, however, must be understood as a relative term, not as signifying an intimately co-adapted host-pathogen relationship. Immunological studies have confirmed the long-standing clinical observation that anthropophilic *T. mentagrophytes* infections are significantly more inflammatory in nature than *T. rubrum* infections of the same body sites [34,35]. The adaptation of *A. vanbreuseghemii* lineages to human pathogenesis is clearly less well balanced than the sophisticated host-pathogen relationship that has evolved in *T. rubrum*. This is entirely consistent with the degree to which the *A. vanbreuseghemii* isolates have retained the morphological and physiological characters of soil-associated zoophily.

It should be noted that the retention by anthropophilic *T. mentagrophytes* of a level of osmotolerance more commonly found in soil-associated organisms allows it to cause superficial white onychomycosis by invading the highly hydrophobic and cornified keratin on upper surfaces of nails [36]. *T. rubrum*, a much more hydrophilic organism, rarely causes superficial white onychomycosis (and then typically in cases where adjacent toes overlap the affected nail surface), and instead typically causes subungual onychomycosis by invading nail beds from below, after growing across well hydrated toe skin to the nail [36]. It then stimulates hyperkeratosis, resulting in a thick nail with a mass of relatively weakly structured, friable material on its underside. *T. mentagrophytes*, in retaining a distinctive niche with low water activity on the human body, may thereby retain selection of at least some of its soil association characters, which were originally selected through association with similarly dry off-host keratin.

Conidia of the substrate mycelium

While infecting animal hosts, dermatophytes, so far as is known, never produce the aerial conidial types. They do, however, produce copious swollen cells in the substrate mycelium. These cells, although they may remain attached to each other and to hyphae in the manner of chlamydospores, may also dehisce by an enzymatically assisted rhexolysis in a manner resembling that by which aerially conidia dehisce, except that discrete empty disjunctors are not seen. These conidia, which will be called substrate arthroconidia to distinguish them from the completely biologically different aerial arthroconidia (which appear and function as intercalary microconidia), have been shown to be the normal infective propagules of the dermatophyte species where this matter has been studied [26, 37]. As infective propagules, they are biologically sophisticated, producing molecules which engage them in lectin like surface- binding interactions specific to the most vulnerable host body sites [26, 37].

The infectivity of microconidia and macroconidia has not yet been studied in the zoophilic or geophilic der-

matophytes. It seems likely, however, that the soil-associated zoophiles, at least, must have a life cycle involving two major habitats, the infected animal, and the shed keratin in and near the animal's dwelling place, the locus where dermatophyte sexuality occurs. Since it is extremely unlikely that off-host growth and sexuality are evolutionary dead ends in these fungi, it is reciprocally highly likely that the inoculum developed on off-host keratin, including that produced as sexual progeny, is potentially infectious. Since much of this off-host inoculum amplification is likely disseminated as macroconidia, microconidia and/or ascospores, it seems highly likely that most or all of these structures would possess the ability to re-establish infection. If this were not the case, sexuality would be of no evolutionary advantage in animal-associated species and, as a complex process likely requiring coordinated performance of multiple genetic loci, would rapidly be lost from all lineages retaining animal association. The relative infectivity of the various spores and conidia produced by geophilic and soil-associated zoophilic dermatophytes has not yet been tested, but easily could be using existing animal models.

In anthropophilic and hoofed animal dermatophytes, where sexuality is deprived of its theatre and amplification of off-host inoculum is of little potential advantage (and also tends to conflict with the reduced osmotolerance of these organisms), aerial conidia likely become vestigial, as argued above, and thus lose the potential of evolving characters selected by pathogenic advantage. The substrate arthroconidium, on the other hand, is purely a manifestation of successful pathogenicity, and forms part of the minimal suite of characters that must be retained by asexual isolates parasitizing humans, horses, and ungulates. It is produced in large numbers not just in infected skin and nails, but also in or on infected hair shafts. The two common variants of hair shaft colonization, endothrix and ectothrix, may be fungible and indicative more of ancestry than specific adaptation, but the infectious inoculum potential of hair (or fur) infections is clearly a function of the density in which such arthroconidia are produced.

Several asexual dermatophytes produce fomites with noteworthy endurance in the environment. The best known is *T. tonsurans*, which can produce fomites with infectivity apparently persisting for at least six months in contaminated environments [38, 39]. The infectious potential of *T. verrucosum* fomites on environmental structures such as fenceposts in cattle handling facilities is also well known. Sinski et al. [40], in experimenting with the half-life of stored human skin scraping specimens from active dermatophytosis, found that such specimens could endure for several months if kept dry, and could also withstand exposure to relatively high environmental temperatures. It is likely that in all these cases, the persisting infective structure is the substrate arthroconidium. Whether or not substrate arthroconidia can persistent in the environment as isolated particles is unknown; it is possible that their survival is assisted when they remain surrounded in a matrix of shed host epidermal cells, a situation analogous to the mixed host/fungal mummiform sclerotia of some sclerotiniaceous fungi. Such a situation would maximize the stability of the microhabitat in which dormancy occurred. This question could be directly studied by attempting to infect animal models with purified host-grown arthroconidia exposed to environmental conditions for several months, in comparison with whole infected skin flakes or hairs exposed to the same conditions.

An interesting feature of substrate arthroconidia is that they appear at least in some species to have an ontogenetic connection with macroconidia. In *E. floccosum*, for example, primary cultures often emerge as mostly consisting of inflated hyphae that break up into large round substrate arthroconidia. Colonies or parts of colonies may even be pasty in nature, since the majority of material consists of separated single cells. The culture then undergoes a stage in which some macroconidia form, but often incorporate some swollen cells reminiscent of arthroconidia, as well as some empty cells allowing disarticulation of parts of the macroconidium. Subsequent growth is more filamentous in nature and bears classic well-formed macroconidia in clusters. Later subcultures, at least on rich media such as Sabouraud agar, soon lose both macroconidia and arthroconidia and degenerate as "pleomorphic" white mycelium.

Similar primary outgrowths are seen in some lineages in the *T. mentagrophytes* complex. A particularly dramatic example is *T. simii*, in which the incorporation of substrate arthroconidia into macroconidia yields the distinctive swollen cells or chlamydospores that facilitate presumptive laboratory identification of this species. In fact, the same phenomenon is also seen in other *T. mentagrophytes* lineages in which cultures occasionally first grow out on isolation media as heavily arthroconidial, and then heavily macroconidial isolates.

Certain plesiomorphous lineages in the *T. rubrum* complex show the same pattern. Closely related to classic cottony *T. rubrum*, and sharing the same ribosomal internal transcribed spacer sequence [5], is *Trichophyton raubitschekii*. This fungus differs from *T. rubrum* by producing copious macro- and microconidia, including many rounded microconidia suggestive of *T. mentagrophytes* conidia [41]. It also has other plesiomorphous characters suggestive of its phylogenetic origin amidst the lineages of the *A. benhamiae* clade of the *T. mentagrophytes* complex, such as urease activity and the production of relatively inflammatory lesions in infected humans [42]. Like *E. floccosum*, it often starts out in primary culture as a colony producing large numbers of substrate arthroconidia, which then intergrade with swollen cells in macroconidia, which in turn may in part break up as arthroconidia. The same phenomenon is seen in the closely related *T. kanei* [21], which also has the *T. rubrum* internal transcribed spacer sequence [5]. Related *T. rubrum* isolates which produce fewer substrate arthroconidia in culture also produce correspondingly fewer macroconidia, and vice versa. It appears likely that the genetic information coding substrate arthroconidia may revert in part to macroconidial production in some dermatophytes in artificial culture. That is to say, the production of macroconidia in culture by these fungi may reflect the conservation of genetic programming ancestrally related to production of functional macroconidia in a soil habitat, but currently normally involved in substrate arthroconidial production in infected hosts. *T. simii*, of course, does express a full suite of soil association characters, including sexuality, and probably does have an active soil reservoir in its life cycle. *T. raubitschekii* and *E. floccosum*, however, are purely anthropophilic and have never (reliably) been reported from soils. Their heavy macroconidiation should certainly not be taken to imply the existence of a cryptic soil habitat.

Characters of the soma

There is a strong physiological contrast in the dermatophytes and dermatophytoids between the soil-associated and non-soil species. Some characteristic

physiological features of the soil-associated dermatophytes have already been mentioned, such as their osmotolerance, vitamin independence and urease activity. Vitamin independence, as with osmotolerance and possibly macroconidial production, correlates with invasion of potentially micronutrient-poor keratinous substrates in or on soil. Another character that appears to correlate with this habit of growth is seen in the growth of some species on bromcresol purple milk solids-glucose agar. Soil-associated *Trichophyton* species such as zoophilic *T. mentagrophytes*, *T. ajelloi*, and *T. terrestre*, as well as relatively plesiomorphous asexual derivatives such as *T. equinum*, begin to produce an alkaline reaction in the bromcresol purple indicator within three to four days of inoculation, showing that they are consuming milk proteins as a carbon source and excreting the surplus ammonium ion this process generates [31]. The anthropophilic *T. rubrum*, however, is catabolite-repressed by the glucose in the medium and does not start degrading the proteins until a considerable portion of the glucose is exhausted, after about 10 days of growth. In the same medium made without glucose, it begins degrading proteins immediately. Unlike the constitutively protein-degrading *T. mentagrophytes* group, it has become sufficiently adapted to the diversity of metabolites made available by the host to be able to turn off much of its potentially immunogenic proteolysis while small nutrient molecules are available [43,44]. The situation of *Microsporum* species in relation to this medium is more complex and the unpublished results relating to this matter will not be discussed here.

The function of urease enzyme in habitats of soil-associated dermatophytes has not been studied. Most ground-dwelling animals take some pains to deposit bodily wastes outside the dwelling or in special midden chambers. It is possible that enough hair and skin are deposited in rodent middens to make this a good dermatophyte habitat; however, this matter has not been investigated. Also, sufficient urea may still be carried into or deposited on (e.g., in territorial markings) other parts of the dwelling, or near the dwelling entrance, to make a significant area of urea-rich dermatophyte habitat. Certainly, the geophiles often found growing on hair-laden carnivore dung (see above) may be at an advantage to possess an active urease. It should be mentioned that it is not known to what extent, if any, dermatophyte ureases may catabolize compounds other than urea. Many soil-associated organisms in general, e.g., soil-dwelling basidiomycetous yeasts, possess a urease enzyme, and its contribution to fungal nutrition in nature is not well understood.

As the soil habitat is abandoned by anthropophilic and hoofed animal dermatophyte lineages, urease appears to be of little advantage and is lost or attenuated in several clades. In the lineage containing *T. soudanense*, *T. megninii*, *T. rubrum*, *T. raubitschekii*, and *T. violaceum* [4,5,19], urease is lost in most *T. soudanense* and *T. rubrum* isolates, weak in *T. megninii and T. violaceum*, and very weak but present in some *T. rubrum*. Only *T. raubitschekii* and some *T. soudanense* isolates retain a fully active urease. Interestingly, *T. mentagrophytes* var. *erinacei*, the hedgehog dermatophyte, while otherwise possessing the standard soil association characters, lacks active urease. Since hedgehogs in summer typically dwell in sleeping places for only a few days, possibly returning to the same places again only after several weeks, and since even in winter they may change their hibernacula (hibernating nests) several times, they may not deposit sufficient urea in or around their nests to selectively reinforce the urease enzyme. Burrowing and denning animals with more stable dwellings, and even wild swine with their regular thicket

retreats and riparian wallows, may provide greater reinforcement for this capacity.

Other dermatophyte species always or often negative for urease — *T. verrucosum*, *M. audouinii*, and *M. ferrugineum* — are all typical non-soil-associated species.

On the whole, the capacity to manufacture vitamins and other growth factors may be relatively expensive or genetically complex, and therefore relatively likely to suffer a disruption in the absence of constant selection over evolutionary time. Of the non-soil dermatophytes, three separate lineages (according to sequence studies) of *T. mentagrophytes* complex descendents, *T. verrucosum*, *T. tonsurans* and *T. violaceum*, have lost thiamine- producing capability. Of other dermatophytes derived from common ancestors of the *T. mentagrophytes* complex one (*T. equinum* var. *equinum*) has lost nicotinic acid synthesis, one (*T. verrucosum*) has in most isolates lost inositol synthesis, and one (*T. soudanense*) has in many isolates lost some growth factor synthesis capabilities, sometimes nicotinic acid and sometimes uncharacterized. In addition, one lineage derived from the *T. mentagrophytes* complex — *T. megninii* — has lost L-histidine synthesis. In all cases, these dermatophytes are non-soil species which can depend on constant host colonization, and an absence of a growth stage in the life cycle involving off-host keratin, to ensure supply of the required factors.

The *M. canis* lineage, which has independently evolved the non-soil species *M. ferrugineum* and *M. audouinii*, has no members auxotrophic for vitamins, as seen on vitamin-free casamino acids agar, but *M. audouinii* does show a requirement for an unknown growth factor in the polished rice test.

Another biochemical character which should be mentioned in connection with ecological function is pigmentation, that is, the production of coloured compounds, typically naphthoquinones. These compounds are typical secondary metabolites of dermatophytes, along with lactam and fusidane antibiotics [45,46]. They are seen as bound or, uncommonly, diffusing pigments giving artificially grown colonies their characteristic colours. Such pigments have never to my knowledge been observed in colonized natural substrata, including infected skin and nails. Our recent studies, however (unpublished) have shown that the *T. rubrum* naphthoquinones xanthomegnin and viomellein do occur in readily measurable quantities in most colonized nail and skin samples.

Classic work showed that most dermatophytes produce a mixture of pigments [47], which together compose the characteristic colony colour. Differently coloured dermatophytes may simply have different ratios of the same compounds. These quantitative colour characters, then, may be fungible; certainly their relative constancy within most species is interesting and has no known functional explanation. The functions, however, of many fungal secondary metabolites are poorly known, and there has been a long history of debate on this issue. Although some secondary metabolites clearly have import in interference competition, e.g., antibacterials, antifungals, nematicides, immunosuppressives, etc., and some may be involved in fungal self-signalling, e.g., "staling compounds," there are many which to date are functionally unaccounted for, and some authors have proposed that they represent functionless metabolic overflow. Indeed, this was the "classical" explanation for both plant and fungal secondary metabolites [48]. Their high degree of qualitative and/or quantitative difference between different fungi growing with equal apparent success in the same microhabitats tends to suggest that a high specificity of adaptation may not obtain in every case.

Dermatophyte pigments may have specific but unknown functions in pathogenesis or microbial competition. Xanthomegnin, for example, is thought to be a kidney toxin when ingested by animals after production in feed by *Penicillium* or *Aspergillus* spp [49]. It seems unlikely, however, that dermatophytes producing small quantities of this compound in infected sites are capable of damaging host kidneys; that they should be specifically adapted to do so is all the more unlikely. Of course, local immunomodulatory effects are possible in colonized epidermis; this question is unexplored. There may be specific effects adapted to protect against particular antagonistic skin flora. Many fungal naphthoquinones generate potentially antagonistic superoxide and hydrogen peroxide when they interact with electrons from the respiratory metabolism of competitors [50]. It is also possible that at least some of the secondary metabolites formed by dermatophytes may lack specific function, either because they are vestigial or because many secondary metabolites in general are (according to some authors) of minimal utility. The present author, however, would like to suggest a third possibility for consideration, and that is that such compounds may have a necessary, nonspecific function. Secondary metabolites are produced as a fungus is maturing in a colonized area. Conidiation or sporulation often co-occurs with the generation of these metabolites [51]. Any still-extending mycelial growth fronts are distally colonizing fresh material in areas still free of secondary metabolism, and in the zone of secondary metabolism, propagules or resting cells are prepared to transport the fungus to altered conditions through space or, in the case of chlamydospores, time. At the same time, hyphae are differentiating in a process terminating in senescence and death. Such a decline vitiates the organism's ability to mount an active defence of its *shiro* [52] or colonized territory, and the possibility emerges of a vigorous decay by bacterial or fungal necrotrophs, which in turn may threaten the propagules. Such an exposure of weakened fungal cells would also self-reinforcingly amplify any capabilities for antifungal pathogenesis associated with the aggressive decomposers (e.g., "mycolytic" or fungus-lysing pseudomonads), which would even be able to attack the fungal mycelium from the rear through the medullae of its moribund hyphae proximal to the colony origin. To mount specific defenses against all these marginally deleterious organisms would require an improbably complex elaboration of inhibitory chemistry. It seems possible, therefore, that an efficient alternate adaptation would simply be to produce a small array of odd, distinctive compounds that most non-specific organisms would lack the ability to assimilate. In short, at least some secondary metabolites, well known for their extensive diversity and chemical distinctiveness, may simply differentiate growth sites as "not recognizable as food." Of course, of the diversity of anomalous and poorly metabolically accessible compounds produced by a fungal species, some may develop and be selectively reinforced in specific adaptive roles, often directly antagonistic [53]. But specific roles elicit specific responses, as is seen in gene-for-gene relationships of parasite virulence and host resistance genes in phytopathology, and this type of "host resistance" [54] is in many ways less robust than the nonspecific "non-host resistance" that protects plants against the generality of nonspecific opportunistic pathogens. Secondary metabolites may be a common way in which fungi secure this broad-ranging nonspecific resistance. Some molecules may have a dual inhibitory function, specifically inhibiting one or more common antagonists through an adapted inhibitory mechanism, while functio-

ning as non-specific inaccessible compounds non-stimulatory to the growth of other, less co-evolved organisms.

The compounds participating in this nonspecific deterrence process succeed in conferring advantage when they are not so prevalent that many opportunist-decomposers develop enzyme systems able to handle them. There is, therefore, a selective pressure for diversity and relatively rapid evolutionary change. This pressure is quite different from the clines in specific selective conditions that give rise to so-called "diversifying selection," as when a plant species grows as a genetically specified range of different size classes according to varying exposure conditions across a gradient of elevation. In non-host resistance, the pressure is not to be specifically different, but simply to be different, nonspecifically anomalous. Any oddly novel compound, alien to enzyme systems of competitors, that happened to arise as a result of genetic change would confer greater advantage than any more environmentally familiar and readily biodegradable compounds. The type of selective pressure so exerted might be called "anomalizing selection." One unrecognized and indigestible compound is as good as another, and a lineage's metabolic history in the production of unusual metabolites is probably the main predictor of what sort of compound will be produced in great excess during secondary metabolism. These compounds, therefore, are both functional and fungible.

The predators or grazers of soil-dwelling dermatophytes include bacteria, protozoans, and arthropods. Those of non-soil dermatophytes are probably mainly bacterial skin flora. The difficulty of isolating dermatophytes from heavily pseudomonad-colonized nails has long been known, and reference laboratories like that of the present author not infrequently receive referred cultures consisting of dermatophyte inoculum which has been rendered non-viable by massive attack of antibiotic-polyresistant, mycolytic bacteria, which may be motile. With their extended persistence in the host skin and nails, anthropophilic dermatophytes may be relatively susceptible to having their nonspecific defenses overcome by specifically adapted bacteria. It is very likely, however, that the metabolic "octopus ink" of unusual heptaketide naphthoquinones supplements the activity of the more specific lactam and fusidane antibiotics by rendering substrate metabolically foreign to the majority of nonspecifically antagonistic skin organisms. When antibiotic production engenders nearby populations of antibiotic-resistant bacteria, as Youssef *et al.* [55] found with *T. rubrum*, the shifting array of quinone pigments may still act as a non-lethal, non-growth-supporting deterrent.

Such anomalizing selection, if indeed occurring, makes it advantageous for different dermatophytes to produce different pigments or ratios of pigments, in order to present as anomalous as possible a chemical interface to opportunistic decomposers. Dermatophyte species come in a wide variety of colony colours, ranging from lemon yellow to orange-brown to blood red to white. The large number of dermatophytes lineally derived from the *T. mentagrophytes* common ancestors are quite closely related, and may differ more in ratio than in type of compounds. The few existing studies appear to support this, although more studies are certainly needed.

It must be borne in mind that biological efficiency often has individual elements performing a variety of tasks, and the enhancement of pigment production by increased salt concentration in some dermatophytes such as *T. mentagrophytes* [24, 56], but not in others such as *T. rubrum*, needs to be kept in mind. Among their other functions, the naphthoquinones and relatives may be

among the compounds that osmotolerant dermatophytes store to increase their own internal water binding potential when the external water activity is strongly reduced. Serving in this role certainly would not negate the metabolic smokescreen role proposed above; the two might be quite complementary.

That pigment production is in some way constantly selected for in pathogenicity is suggested by the rapid loss in cultural degeneration in some species, most notably *T. violaceum*. This fact alone should also prompt some study on the topic of whether such compounds may be immunomodulators, activated oxygen quenchers, or other types of virulence factors.

Various other potentially immunomodulatory or immunogenic components of dermatophyte chemistry have been investigated, but discussion of their form and function is beyond the scope of this review.

Some morphological adaptations of the soma also are significant contributors to the uniqueness of dermatophyte biology. Hyphae in this group of fungi produce a number of distinctive features [22,23], including perforating organs, pectinate (closely spaced unilateral) branching, frondose branching, racquet hyphae, "propagules", nodular bodies, filiform branching (e.g., "caterpillar forms" of *T. tonsurans*), reflexive branching, and diverticula or hyphal projections. Some of these structures are clearly related to physically assisting the enzymatic penetration of keratin. Perforating organs are a soil association character, found almost entirely in dermatophyte species in which at least some members retain a sexual, soil-dwelling state in their life cycle. They are clearly related to the penetration of off-host hairs, and possibly other environmental keratinous structures (e.g., feathers); they are never seen in growth on infected hosts. On the other hand, frondose branching, in which hyphae expand into flattened, laterally expanded structures with irregular finger-like projections, is commonly seen in growth of *T. mentagrophytes* on human nails [36], and represents a maximization of the catalytic surface area spreading between horizontal layers (strata) of host nail keratin. Pectinate branching, which mainly consists of regions of hyphae giving rise to a "comb-like," more-or-less planar series of unilateral projections, may be related. It, however, like the formation of racquet hyphae, is a character found in Onygenalean fungi outside the Arthrodermataceae, sometimes in the mycelium but sometimes in specialized appendages around ascomata. The genus *Ctenomyces* is well known for its pectinate appendages. It may, therefore, be a vestigial remnant of a once functional morphogenetic pathway apomorphic within the Onygenales, and may lack a function in human and animal pathogenesis. Or, perhaps again like racquet hyphae, it may be a fungible hyphal growth character indicative of nothing more than a viable alternate way of differentiating functional hyphae. The present author has isolated a physiologically normal but morphologically unusual, nonsporulating *T. rubrum* strain from a human dermatophytosis lesion. The main distinctive feature of the strain is that it grew more-or-less entirely by means of pectinate branching (Figure 3). If nothing else, such an aberrant character exemplifies the high amount of genetic drift which is likely to be occurring in morphological ontogeny of asexual, non-soil-associated dermatophytes. Such unusual characters as *T. tonsurans* "caterpillar" structures and "propagules" or "proliferating bodies" of the *T. mentagrophytes* isolates segregated by English and Stockdale [57] as *T. proliferans* may be artefacts of this drift, along with more mundane characters such as loss of aerial conidiogenesis.
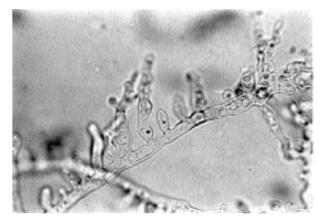

Figure 3. Pectinate vegetative hyphal growth in an aberrant *Trichophyton rubrum* isolate (1000x).

There are a number of ambiguous characters where relation to function, ancestry or morphogenetic drift are possible, but no one explanation seems more likely than the others. Further study of these characters, which have been marked as taxonomic indicators but otherwise not investigated in detail, might yield some clues about their relation to function. For example, there is no clear explanation for the appearance of nailhead hyphae in *T. schoenleinii*. These hyphae somewhat resemble frondose spreading, which is found *in vivo* in onychomycosis caused, for example, by *T. mentagrophytes*, but in this case occur in an organism that is not known as a major agent of onychomycosis. To my knowledge, nailhead hyphae have only been discerned in culture, not in host material. The thickened lateral hyphal projections of *T. rubrum* may resemble antheridia, and may vestigially represent this otherwise functionless morphogenetic program, but the most closely related sexual species do not form such structures in sexually unstimulated, vegetative mycelium, and it is mysterious that the single-mating-type *T. rubrum* should do so. Perhaps the projections have no connection to antheridia. They also resemble hair perforating organs — but in an organism which does not make functional perforating organs, and out of any contact with hair. Similarly, the nodular bodies of *T. krajdenii* have superficial similarity to coiled precursors of ascoma formation, but develop in submerged mycelium where Onygenalean sexual structures never occur. Moreover, they are full of naphthoquinone pigments, a character more typical of submerged mycelial structures than aerial phase reproductive structures in dermatophytes. Finally, the reflexive branching of *T. soudanense*, a very unusual character in the fungi where the tendency of mycelium is almost always to proliferate radially outward toward uncolonized areas, may be related to interlamellar spreading in keratin, or may be explained as another example of morphogenetic drift, or may require a novel explanation which can only arise from further research. One generalization that can be made about many such ambiguous structures is that they do mostly occur, or at least have mostly been noted, in asexual dermatophytes where morphogenetic drift, often seen as attenuation of conidiation, is otherwise known.

Conclusion

The science of dermatophytes in recent years has become strongly focussed on a small number of questions: dermatophytes' phylogenetic origins and taxonomy, their immunological and virulence relations, their enzymes,

their epidemiology in the clinic and in soils and human habitations, and their laboratory identification. The present review should indicate not only how many poignantly unanswered questions there are about other aspects of the biology of these fascinating organisms, but also should give a sense that there are areas of meaningful scientific inquiry that have been entirely left out of the "gaze" (that is, the publicly legitimized realm of consideration) of dermatophyte mycology. A good example is the relation between dermatophyte structures and arthropod grazers. Not all the biologically relevant questions that could be asked have immediate practical import, and that may partially explain the inhibition, but one hopes that the value of pure biology, and its potential for reconnecting with practical biology, will continue to be recognized. Perhaps the day will come when an astute investigator will actually record macroconidial mycelium in direct microscopic examination of inner wall material from a rodent burrow, or pectinate branches on the surface of a shed cat hair. Certainly, as progress in morphogenetics increases, assisted by concerted sequencing, the probability of finding the underlying, efficient connections between structures like substrate arthroconidia and macroconidia increases. Until then, the existing evidence resolves some questions, and assists our understanding of the fundamental division between sexual, soil- associated and asexual, non-soil-associated members of the Arthrodermataceae.

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