Clues to the presence of pathogenic fungi in certain environments

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The presence of various pathogenic fungi in rather unsuspected hosts and environments has always attracted the attention of the scientific community. Reports on the putative role of animals in fungal infections of humans bear important consequences on public health as well as on the understanding of fungal ecology. Fungi are ubiquitous in nature and their great capacity for adaptation allows them to survive and indeed, to thrive, in plants, trees and other natural substrata. Nonetheless, we are just beginning to learn the significance that these diverse fungal habitats have on the increasing number of immunosuppressed individuals. The accidental or permanent presence of fungi in animals, plants, soils and watercourses should not be taken too lightly because they constitute the source where potential pathogens will be contracted. If those fungal habitats that carry the largest risks of exposure could be defined, if seasonal variations in the production of infectious propagules could be determined, and if their mode of transmission were to be assessed, it would be possible to develop protective measures in order to avoid human infection. Additionally, unsuspected avenues for the exploration of fungal survival strategies would be opened, thus enhancing our capacity to react properly to their advancing limits. This paper explores several ecological connections between human pathogenic fungi and certain animals, trees, waterways and degraded organic materials. The occurrence of such connections in highly endemic areas will hopefully furnish more precise clues to fungal habitats and allow the design of control programs aimed at avoiding human infection.

Keywords armadillos, bamboo rats, jungle trees, pathogenic fungi

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Animals and their connection with human pathogenic fungi

The bamboo rat and Penicillium marneffei

In 1955, several investigators in Vietnam were attempting to establish an animal model of rickettsiosis in a bamboo rat. Following the inoculation of rats with *Rickettsia orientalis*, three animals suddenly died from a fulminant infection of the reticuloendothelial system [1]. Autopsy of the dead rats indicated that a yeast-like organism was the probable cause of death. However, surprise ensued when culture of the rats' infected organs yielded a pure mould growth of a *Penicillium*, a genus not traditionally considered pathogenic for humans or animals. Therefore, the fungal isolate and a laboratory mouse inoculated with the putative pathogen were sent to Dr Gabriel Segretain, at the Pasteur Institute in Paris who identified the fungus as a new species and gave it the epithet *Penicillium marneffei* [2,3].

From its discovery until the late 1980s, *P. marneffei* remained relatively obscure and was considered more of a mycological curiosity rather than a serious pathogen until the acquired immune deficiency syndrome (AIDS) pandemic dramatically changed this image. The first cases of penicilliosis due to *P. marneffei* among confirmed human immunodeficiency virus (HIV)-positive individuals were reported in 1988 [4–6]. Since then, this species has infected over 1600 AIDS patients throughout Southeast Asia where this fungus is geographically restricted. Penicilliosis due to *P. marneffei* is now recognized as an AIDS-related indicator disease in this region [7]. Interestingly, the rise in worldwide HIV infection has not led to parallel increases in the incidence of penicilliosis by other *Penicillium* species despite their global distribution. Only four cases involving different species have been recorded in AIDS patients [8–10].

The emergence of *P. marneffei* infections has had an enormous impact upon people in Southeast Asia. At Chiang Mai University Hospital in Northern Thailand, 14–16% of AIDS patients contracted penicilliosis resulting in more than 1300 cases of this disease since 1990 [11–13]. This ranked penicilliosis caused by *P. marneffei* as the third most frequent opportunistic infectious disease of this patient group, surpassed only by tuberculosis and cryptococcosis. A smaller hospital in Chiang Mai reported that 20% of AIDS patients acquired a *P. marneffei* infection [14], whereas 144 cases were documented during a 1-year period in neighboring Chiang Mai [15,16]. The prevalence of penicilliosis outside this area has been slightly less, but significant. For example, 5·8% of Bangkok (Southern Thailand) AIDS patients became infected with *P. marneffei* [17]. In Hong Kong (Southern China), *P. marneffei* infections were the third most frequent AIDS-defining illness (9·4%), following only *Pneumocystis* pneumonia and tuberculosis [18,19]. More recently, the epidemic has expanded to include autochthonous AIDS patients in Taiwan and India [20–22].

Curiously, the medical literature indicates that only 36 cases of infection due to *P. marneffei* have been noted outside the endemic region. All patients had previously visited or lived in Southeast Asia. Given the ubiquitous nature of *Penicillium* species [23] and the rampant HIV pandemic, the question remains as to why *P. marneffei* infections have been limited solely to this part of the world? Initially, some investigators believed that the answer rested in the connection with the bamboo rat.

P. marneffei carriage rates in bamboo rats

The association of the bamboo rat and *P. marneffei* is uniquely intriguing to medical mycologists. Besides humans and bamboo rats, no other animal is naturally infected by this pathogen. A reasonable deduction is that the fungus is part of the normal flora inhabiting the soil and surrounding vegetation. Bamboo rats, then, would conceivably encounter *P. marneffei* as part of their natural habitat. In addition, because bamboo rats are frequently associated with human dwellings and also serve as a food source for some of the indigenous human population, connections among the rats, fungus and human disease seems plausible. However, in reality, this triad relationship is far from simple.

Southeast Asia is home to two distinct genera of bamboo rats (Fig. 1). One genus, *Rhizomys*, contains three common species: *Rhizomys sinensis*, *R. pruinosis* and *R. sumatrensis*. The other genus is comprised of a single species that serves as a host, *Cannomys badius*, which can be further divided into two subgroups based upon fur color: red-brown and gray-black. While the distribution of individual species vary, collectively they cover an area from southern China and Nepal to Malay and Sumatra.

Since the 1980s, the possible connection between the bamboo rat and disease due to *P. marneffei* prompted several epidemiological investigations in which the carriage rates of the fungus were measured in captured rats [24–28]. The collective results of these studies are presented in Table 1 [29]. Among rats captured in or near southern China, *R. pruinosis* was found to be the more prominent species. Collectively, 66% of *R. pruinosis* were found to harbor *P. marneffei* in their internal organs. Only two rats identified as *R. sinensis* were captured for study, but both carried *P. marneffei*; additionally, three new animals also revealed the fungus. The capture and
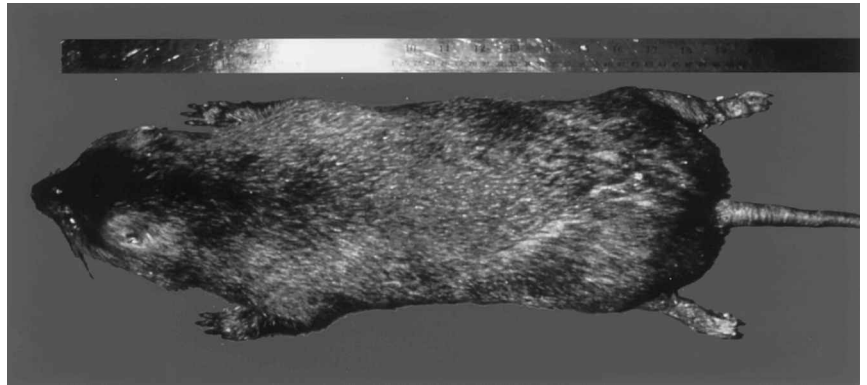
Fig. 1 Bamboo rat captured from a field study in Thailand. The ruler indicates the body length of the rat is approximately 480 mm. Photograph courtesy of Dr Konrad Nelson.

study of *C. badius* was not reported in these investigations. However, more recent studies focusing on the infection rate of bamboo rats, including both types of *C. badius*, were conducted in Thailand. Investigators reported that 12 of 92 (9·8%) *C. badius* rats autopsied carried *P. marneffei* as did six of eight (75%) of *R. pruinosis* and 13 of 14 *R. sumatrensis* rats. Interestingly, in the one study that distinguished *C. badius* rats based upon fur color, *P. marneffei* was not cultured from any of the 51 gray-black individuals examined.

In an attempt to correlate specific strains of *P. marneffei* with the host animal from which it was cultured, Vanittanakom *et al*. [30] determined the DNA restriction endonuclease patterns of selected isolates. Two DNA types (type I and type II) were identified. Human-derived isolates of *P. marneffei*, occurring among patients diagnosed in Chiang Mai, Thailand, were comprised of both type I and II strains. Interestingly, all 20 isolates cultured from 12 individual *R. sumatrensis* rats were type I strains, whereas all three isolates from three different *C. badius* rats were type II strains. The lone soil isolate obtained from the burrow of a *R. sumatrensis* rat was also type II. These results represent the first molecular data for penicilliosis caused by *P. marneffei*, but do little to clarify the role of the bamboo rat.

The collective data suggest that the bamboo rat is often infected by the fungus, thereby serving as a possible vehicle for transmitting the disease to humans. However, case control studies in Thailand could not establish exposure of a susceptible person to a bamboo rat as a risk factor for acquiring penicilliosis despite the close proximity of human and rat habitats [31]. Neither could the consumption of the bamboo rat as food be linked to disease. Rather, the critical risk factor appears to be exposure to soil, particularly during the rainy season [31,32]. This latter suggestion presents a curious paradox. Numerous attempts to culture *P. marneffei* from soil or vegetation have been unsuccessful. In fact, other than the

isolation of the fungus from rat or human specimens, *P. marneffei* has only been cultured on very rare occasions from bamboo rat burrows [29]. Clearly, rats and humans acquire infection by *P. marneffei*, but the reservoir and mechanism of transmission remains an enigma.

Alternative suggestions for a P. marneffei reservoir and disease transmission

The bamboo rat does not appear to be a reservoir or means of disease transmission for *P. marneffei*. Rather, the animal seems to be a sentinel indicator. This leaves an enormous gap in our understanding of the ecology of *P. marneffei* as well as the epidemiology of penicilliosis caused by this fungus. Hence, alternative approaches must be considered if the natural reservoir of *P. marneffei* is to be discovered.

An obvious consideration is that another animal serves as the true reservoir. However, there are no recorded attempts to examine other animals for the presence of *P. marneffei*. It would seem to be a reasonable step to perform field studies on selected animals that are associated with humans and their environment.

Other possibilities relate primarily to the effects of the local environmental conditions upon the dissemination of *P. marneffei* in nature. For example, changes in the climate as well as land use can conceivably alter the

Table 1 Frequency of *P. marneffei* isolated from bamboo rats*

Organism	Frequency of isolation
Rhizomys siensis	100% (n = 5)
R. pruinosis	66·4% (n = 244)
R. sumatrensis	92·9% (n = 14)
Cannomys badius	9·8% (n = 92)

*Collective results of seven different field studies. Adapted from Cooper [29].

ecological niche of the fungus. Depending upon the particular response of *P. marneffei* to these changes, infectious propagules could be dispersed or not produced at all. Hence, a more global survey involving different types of researchers may play a critical role in establishing the reservoir of this pathogen. Such a survey might include satellites orbiting the earth gathering climatological and geographical data that could be compared to previously obtained information. Correlations might then be drawn to the incidence and location of penicilliosis, which may help establish the general parameters of a *P. marneffei* reservoir.

Finally, the biology of the organism itself should be studied in greater detail. Such investigations might lead to physiological clues that pinpoint the conditions necessary for survival of the fungus in a particular environment. For example, a better understanding of the dimorphic nature of *P. marneffei* could provide important insights. The dimorphism of *P. marneffei* is expressed in the formation of arthroconidia from the mould phase. This morphogenic process can be induced *in vitro* by culturing the fungus at 37 °C [33,34]. Because their development appears requisite for the pathogenesis of *P. marneffei*, arthroconidia may actually serve as the infectious propagule. Hence, detailed knowledge of arthroconidiogenesis, combined with ecological knowledge of the endemic area, may direct investigators to the reservoir of *P. marneffei* in nature.

Nine banded armadillo *Dasypus novemcinctus* and *Paracoccidioides brasiliensis*

Paracoccidioidomycosis was first described in l908 [35] but certain aspects of the etiological agent, the dimorphic fungus *Paracoccidioides brasiliensis*, remain poorly understood. Prominent among these is the lack of information concerning the precise habitat of the fungus and the circumstances leading to production of its infective propagules [36]. The prolonged latency of the disease, the frequent migration of the inhabitants of the endemic area, and the lack of reports on possible outbreaks hinder defining the whereabouts of the fungus in nature [36,37]. On rare occasions it has been isolated from soil or soil related products, and from the feces of both frugivorous bats (*Artibeus lituratus*) and a penguin (*Pygoscelis adeliae*). More recently, the nine-banded-armadillo (*Dasypus novemcinctus*) has been shown to carry the fungus in its internal organs and this mammal is currently regarded as another host to the fungus [38–51]. The latter finding is opening new avenues in the search for the natural habitat of the fungus [45–52].

Nine-banded armadillos inhabit an extensive region of the Americas, from South Central USA to Argentina, along the west of the Andean ridge, a distribution that partially coincides with that of human disease [36]. The armadillos have frequent contacts with soil, particularly when digging, carrying litter for roosts, drinking from streams and foraging [53,54]. These activities probably account for their exposure to infectious aerosols. Armadillos have a low body temperature, a weak cell-mediated immunity, and have been previously indicated as model organisms for several other infectious diseases [55,56].

P. brasiliensis *infection is common and widely distributed in the nine-banded armadillos*

The initial report by Naiff *et al.* [45] on the occurrence of *P. brasiliensis* in nine-banded armadillos captured in the Brazilian Amazonian forest attracted the attention of other workers. Studies aimed at defining the interaction between the fungus and *D. novemcinctus* were then carried out. Two groups in Brazil [47,48,51] and one in Colombia [50] have approached the problem; however, Bagagli and coworkers [47,48] have had the largest experience with their studies focusing on a highly endemic area for paracoccidioidomycosis in Botucatú, São Paulo State, Brazil. Fifteen adult nine-banded-armadillos (eight male, seven female) were captured under a license from the Brazilian Federal Environmental Protection Agency. Four different counties in Botucatú's neighborhood (Botucatú, Manduri, Pardinho and Pratânea) represented by 12 sites served as capture areas. Soil samples ($n = 37$) were also collected and subjected to animal inoculation.

The plating of a large number of small fragments (100–1000) from lungs, spleen, mesenteric lymph node and liver [47] enabled the isolation of *P. brasiliensis* in 66·6% (10/15) of the armadillos. Additional evidence of active fungal disease was obtained by histopathology in three of these animals, thus showing that they may also develop active paracoccidioidomycosis. Regional distribution of positive and negative animals proved heterogeneous and depended on the county; for instance, in Manduri the four armadillos were positive, while in Pardinho the three animals studied proved negative. The proportion of positive armadillos in the remaining counties surveyed varied 66–80%. The fungus was isolated from different organs especially from mesenteric lymph nodes and the spleen, and to lesser extent from liver, both in male (70%) and female (57%) animals. This indicates that in armadillos *P. brasiliensis* disseminates throughout the body such as it does in man [35]. Table 2 presents the data corresponding to infected armadillos recorded in the

Table 2   Natural infection by *Paracoccidioides brasiliensis* in the nine-banded armadillo *Dasypus novemcinctus*. Modified from Corredor *et al.* [50]

First author [Ref.]	Area of capture State, Country	Total animals studied (*n*)	Positive animals (*n*)
Naiff [45]	Pará, Brazil	20	4
Naiff [46]	Pará, Brazil	29	18
Bagagli [47,48]	São Paulo, Brazil	15	10
Macedo [49]	Goiás, Brazil	5	2
Corredor [50]	Caldas, Colômbia	3	1*
Silva-Vergara [51]	Minas Gerais, Brazil	21	1
Total		93	36
Percent of positive animals			38·7

*, In this study, polymerase chain reaction (PCR) allowed amplification of *P. brasiliensis* DNA in this animal as well as in another two that were culture-negative [50].

literature. Surprisingly, soil samples collected around the positive armadillos' burrows proved negative by inoculation into hamsters, an animal susceptible to the infection by this fungus [47].

The *P. brasiliensis* isolates from armadillos have been extensively characterized in terms of virulence, antigens and molecular aspects, resulting in strong evidence that the same 'ecopathogenotypes' can infect both human and animal [47,57,58].

Environmental features in the study sites
Positive armadillos were more frequently associated with sites near water sources and where the vegetation had been highly disturbed [47]. In areas with positive animals, different plants and trees were found, among them the non-autochtonous *Pinus* and *Eucalyptus*; there were also forests, savanna lands, and both semideciduous tropical and riparian forests. The altitudes were below 800 m and the medium temperature fluctuated 14·8–25·8 °C. Soils differed in their composition (sandy or clay), pH varied 3·9–6·0 and the fertility was also diverse. In contrast, in the county (Pardinho) where all armadillos had proven negative, the altitude was higher (950 m), the vegetation, a semideciduous tropical forest, was preserved and water sources were scarce. With the exception of altitude, other studies also described the presence of ecological factors similar to the ones referred to above [50,51].

Armadillos: hosts, reservoirs or mere bystanders?
Would the armadillo play a more significant biological role than that of an accidental host and/or a sentinel indicator of the presence of *P. brasiliensis* in nature? Would other animals sharing both the habitat and the digging habit of the armadillos be infected? Would the *P. brasiliensis* life cycle include an obligate host (such as the armadillo) that would help the fungus to survive the hardships of a changing and competitive soil environment?

The natural habitat of *P. brasiliensis* has proven elusive and the isolation of this fungus from environmental samples is still uncommon [36]. The question is, therefore: has the fungus found in the armadillo a better habitat where it would be free of the hazards of maintaining an environmental microniche? But if so, what is the link to human infection? There are still a number of unresolved questions, essential to a better understanding of the ecology of this dimorphic fungus. Nonetheless, the new findings are offering the opportunity to look more closely at the intricate relationships between the fungus, the armadillo and human paracoccidioidomycosis.

Relationship of *Cryptococcus neoformans* varieties with jungle trees

Environmental studies on *Cryptococcus neoformans* began in the 1950s when Emmons [59] found that *C. neoformans* var. *neoformans* was associated with weathered pigeon droppings and nests. The cosmopolitan character and ubiquity of this variety in disturbed environments, mostly urban areas, was disclosed in successive investigations. In contrast to *C. n.* var. *neoformans*, *C. n.* var. *gattii* has never been found in organic nitrogen-rich substrata and only recently has its microniche been detected.

Searches directed towards plant materials as previously suggested by Staib *et al.* [60], and Bauwens *et al.* [61] led to new environmental findings and shed light on the complex ecology of *C. neoformans*. In 1990, Ellis & Pfeiffer [62] pointed to the association between *C. n.* var. *gattii* serotype B and eucalyptus trees in Australia, thus establishing its natural habitat. Later on, this variety was isolated from the same tree species in other countries [63–65]. Our group studied possible saprobic sources for *C. neoformans* in Rio de Janeiro, Brazil [66] and in 1996 a new natural habitat for *C. n.* var. *neoformans* was

described and related to decaying wood in hollows of living trees [67]. In 1998, Callejas *et al.* [68] described the isolation of *C. n.* var. *gattii* serotype C in tropical almond trees (*Terminalia cattapa*) in Colombia.

Brazilian trees and C. neoformans

Brazil is a large continental country, originally covered by dense tropical forests or jungles. In the northern region, the Amazonian Forest encompasses partially preserved jungle that maintains wild areas with rare and localized urban settlements. In the northeast, the humid coastal area is covered by the remains of an Atlantic forest-type vegetation, and the central semi-arid region or 'caatinga' is covered by brushwood. The western portion of the northeast comprises the states of Piauí and Maranhão and represents a transitional area between the Amazonian forests and the brushwood region. The central-west region, mostly covered by savanna forests, is partially preserved. In the southeast region, only 7% of the original vegetation of the Atlantic forest remains in existence while the jungle has been destroyed to accommodate an increasing urban population. The southern region with Araucarian forest and savannas has also been mostly destroyed. This variety of environmental circumstances prompted the study of *C. neoformans* in Brazilian trees. Botanical data referred to here were obtained from a variety of sources [69–73].

Studies began in southeastern Brazil where samples were collected by scraping decaying wood from the inner hollows of selected trees. The material was plated on niger seed agar medium and the suspected phenol-oxidase positive colonies were identified as previously described [67].

A survey carried out in the urban zone of the city of Rio de Janeiro made it possible to study several trees belonging to different genera, among which the following were shown to harbor *C. n.* var. *neoformans*: Java plum (*Sygygium jambolanum*), native of India and Brazil; November shower (*Senna multijuga*) coming from the Atlantic Forest; fig tree (*Ficus microcarpa*) originally from Malaysia, India and China but widely planted as ornamental shade tree; and pink shower (*Cassia grandis*), a native of the Amazonian forests of Brazil and neighboring countries. The latter tree species were adapted to urban, periurban and rural environments, encompassing areas from north to southeast Brazil. In the city of São Paulo, southeast region, a tree called 'sibipiruna' in the native language (*Caesalpinia peltophoroides*), originally from the Atlantic jungle forest was also found positive for *C. n.* var. *neoformans* [74] (Table 3).

In the northeastern region of Brazil, which is endemic for cryptococcosis due to *C. n.* var. *gattii* [75], the first environmental isolation of this variety was obtained from material collected in the hollow of a pottery tree (*Moquilea tormentosa*) [76]. The same sample inoculated into hamsters produced a disseminated infection with subcutaneous abscesses. Pottery trees are native of this region of Brazil and well adapted to urban environments. In this same area, four pink shower trees were also positive, two sharing both varieties in the same hollow, one positive for *C. n.* var. *neoformans* and the other one for *C. n.* var. *gattii*. A fig tree was also positive for the latter variety [77,78]. For the first time, a common natural biotope shared by the two varieties of *C. neoformans* was discovered in a tree native to jungle forests. In addition, these environmental findings correlate with the occurrence of cryptococcosis in natives, including children, caused by *C. n.* var. *gattii*, observed in the same region [79,80].

In the north, two genera of trees were found positive for *C. neoformans* in a trail of the Amazonian forest: *Miroxylon peruiferum* and *Theobroma cacao* [77,78]. *T. cacao* is a typical jungle tree commercially used for the production of chocolate. This tree was positive for *C. n.* var. *neoformans*, thus disclosing a clear relationship between the fungus, the tree and the jungle environment. *M. peruiferum*, called 'cabreuva' and native to the Atlantic forest, was also found positive for *C. n.* var. *neoformans* in the Amazonian jungle, thus showing the dispersion of native trees from the original areas and their adaptation to other environments (Table 3).

Table 3 *Cryptococcus neoformans* associated to tropical trees according to Brazilian region and variety of the isolate

Brazilian region	Tree (common name)	No. positive trees (*C. neoformans* variety)
Southeast	Pink shower tree	4 (*C. n.* var. *neoformans*)
	Fig tree	2 (*C. n.* var. *neoformans*)
	Java plum	1 (*C. n.* var. *neoformans*)
	November shower	1 (*C. n.* var. *neoformans*)
	'Sibipiruna'	1 (*C. n.* var. *neoformans*)
Northeast	Pottery tree	1 (*C. n.* var. *gattii*)
	Pink shower tree	2 (*C. n.* var. *gattii*)
		1 (*C. n.* var. *gattii* & *C. n.* var. *neoformans*)
		1 (*C. n.* var. *neoformans*)
	Fig tree	1 (*C. n.* var. *gattii*)
North	Cocoa tree	1 (*C. n.* var. *neoformans*)
	'Cabreuva'	1 (*C. n.* var. *neoformans*)
	Circassian seed	1 (*C. n.* var. *gattii* & *C. n.* var. *neoformans*)
	Coral tree	1 (*C. n.* var. *gattii*)
	'Guettarda'	1 (*C. n.* var. *gattii*)
	Java plum	1 (*C. n.* var. *neoformans*)

In Roraima, the northernmost state of Brazil, the city of Boa Vista is located close to the border of the Amazonian forest. Different genera of trees were found positive. The Circassian seed (*Adenanthera pavonina*), a large tropical tree native of Asia, adapted to the Brazilian biotope, and a tree found by the sidewalks of the city, was positive for both varieties that shared the same hollow. The coral tree, or 'mulungu' (*Erytrina velutina*), native of Africa and Brazil, was also found positive for *C. n.* var. *gattii*; and a Java plum was positive for *C. n.* var. *neoformans* [81,82] (Table 3).

Up to this point, all of the environments investigated for the presence of *C. neoformans* had been subjected to certain anthropic action; this was not so in the ecological station of Maracá Island by the Uraricoera river, Roraima State. A native Brazilian jungle tree, *Guettarda acreana* [81,82], was positive for *C. n.* var. *gattii*. Thus, the relationship between a jungle tree and *C. n.* var. *gattii* was demonstrated in a wild area where no anthropic action had taken place.

Anthrophic changes and adaptation of C. neoformans
In Brazil no specific host trees were associated with the *C. neoformans* varieties, which, on the contrary, were found in hollows of different botanical families and species, some native to dense tropical forests in Brazil, and others native to Africa, Asia and Malaysia. The latter had been adapted to the Brazilian biotope and to urban environments as well. The isolation of both varieties of *C. neoformans* from native trees in virgin forests points toward the occurrence of this species in environments where the original vegetation has been preserved.

It is possible that *C. neoformans* life-history strategies involve an origin in decaying wood with successive adaptations to different trees; the latter are dispersed and become adapted to environments where human interference and changes become more and more noticeable. Consequently, environmental disturbances and anthropic action appear to play a significant role in the epidemiology of cryptococcosis, as well as in the ecology of its etiological agent.

The complex ecology of *Blastomyces dermatitidis*

Defining the ecological niche of *Blastomyces dermatitidis* remains a vexing problem. Much has been surmised from rare environmental isolations, and clustered cases. This summary emphasizes new information since the review by DiSalvo [83], including our own investigations in a highly endemic area for blastomycosis in north-central Wisconsin, USA [84–92].

Macroecology

B. dermatitidis is endemic in North America, where it is spreading north and west [93], central India and Africa [83]. Northcentral Wisconsin has the highest reported annual incidence rates of 40/100 000 for humans [85,86], and 1400/100 000 for dogs [87]. The physical geography of highly endemic areas often includes forested podzolic or sandy soils, elevations of 30–575 m (range to 1600 m above sea level) and striking climatic differences over all seasons. Moisture, changing water levels, close proximity to waterways [84,86–88,94] and excavation are associated with infection [87,88]. It is unclear whether the association of waterways with *B. dermatitidis* involves unique physical features of near-shoreline habitats, or rather that waterways serve as gathering places for fauna or flora important to survival of the fungus.

Outbreaks have implicated a variety of ecological sites, including waterways, swampy woodlands, rural sites and urban areas [83,89,95], and have involved excavation, construction, hunting, fishing and other soil related activities. *B. dermatitidis* may be acquired regularly near a place of residence, given the lack of association of outdoor activities with blastomycosis cases [83,85–87,96,97, DJ Baumgardner, unpublished results], and the results of a recent investigation of two households with infected house-confined pets [90]. Of 229 domiciles in our registry of human and dog blastomycosis cases [90], a minimum of 12% were associated with more than one blastomycosis case and seven of 27 with more than three. In most cases diagnoses were separated by one or more years. Recently, we have isolated *B. dermatitidis* from a woodpile located 5 m from a kennel associated with four cases of dog blastomycosis, and 20 m from the owners' house [84]. The organism has also been isolated from between the walls of an abandoned house [83].

Microecology

B. dermatitidis grows on a variety of sterilized natural and manufactured substrates but fails to grow on non-sterilized samples of most of these materials. *B. dermatitidis* mycelial forms are inhibited by certain soil Streptomycetes, bacteria and fungi; yeast phase cells are lysed in non-sterilized soil. The fungus may withstand temperatures of 0–40 °C, including serial freezing and thawing, does not remain viable at 60 °C, but apparently survives the transiently high temperature of a tobacco shed (80 °C) [83]. Some cases are acquired from the environment during months when the snow covers the terrain, and after multiple freeze–thaw cycles [85–87].

There has been much speculation regarding the role of animals in the microecology of *B. dermatitidis*, which is

presumed to be a saprobe; animals could either be providers of specific nutrients favoring its growth, or serve as secondary hosts. Natural infection appears to be restricted to mammals. The fungus has been recovered from the stool of bats [98], and also from a similar sample from a dog with pulmonary blastomycosis [91]. It is unclear if these findings represent a significant mode of spread of the organism in the environment. *B. dermatitidis* has been isolated from environmental sites associated with animal manure and from beaver pond structures [84,85]. However, we have been unable to isolate the fungus from rectal swabs of beavers (*Castor canadensis*) trapped in a highly endemic region [92]. Recently, blastomycosis has been associated with excavation of an abandoned prairie dog colony [93]. Prairie dogs, like many burrowing animals, create specific underground latrine chambers to house their waste. Indeed, most of the suspected environmental venues of this fungus include a variety of ground dwelling animals, some of which practice coprophagy, which results in highly digested wood and vegetation by-products [83, DJ Baumgardner, unpublished results].

Molecular ecology

Long considered a thermal dimorph, *B. dermatitidis* was shown to exhibit nutritional dimorphism as well [99]. We have also demonstrated nutritional conversion to the yeast phase at 21 °C on a simplified medium of allantoin, glycerol, KH_2PO_4, $MgSO_4$, and yeast extract [91]. It has not been determined if nutritional dimorphism could occur at moderate ambient temperature in the natural environment.

The *B. dermatitidis* isolates in our collection utilize allantoin, creatinine, guanidoacetic acid, guanidine and cysteine as sole nitrogen source, but not as sole nitrogen and carbon source. Urea and uric acid were also able to serve as sole nitrogen source with glycerol as the carbon source. Allantoin (a water-soluble end product of purine degradation excreted by most non-primate mammals, turtles and mollusks) in combination with either dextrose, glycerol, lichenen, cellobiose, or xylitol supports fungal growth at room temperature. When both a carbon and a nitrogen source are supplied, *B. dermatitidis* tolerates moderate levels of methanol, alpha-pinene, tannic acid and polyethyleneglycol-200 [DJ Baumgardner, unpublished results]. We are investigating the role of lignin. Therefore, the organism appears to tolerate a number of compounds which might be found among breakdown products of wood or vegetation and also of animal droppings.

Room temperature yeast conversion occurs best on media containing glycerol, allantoin, and yeast extract. Glycerol seems to favor nutritional conversion of the organism compared to dextrose, xylitol, sorbitol and myo-inositol (no growth). Overall, it appears that a variety of inorganic compounds, including nitrogen salts, inhibit yeast conversion at room temperature. Strain differences in fungal growth and yeast conversion at room temperature have been demonstrated [DJ Baumgardner, unpublished results].

Hypotheses

From previous findings it appears that *B. dermatitidis* microfocus includes organic matter that commonly contains rotting wood and/or vegetation, as well as bird or animal droppings; it should be slightly acidic, shaded, at least intermittently moist, and lacking certain biological or chemical inhibitors [84]. The striking infrequency of environmental isolations of this fungus, if not due to the relative insensitivity of our isolation techniques, may reflect its relatively restricted, often transient, microfoci compared to that of *Histoplasma capsulatum* var. *capsulatum*.

The following personal hypotheses may help to explain why *B. dermatitidis* occupies a specific site at a given time:

1. The fungus may be a survivor of rapidly changing conditions of temperature, pH, nutrients, toxic substances, water tension and other factors. In transient microfoci it may thrive temporarily during radical changes in its habitat such as the virtual sterilization of the floor of the tobacco-curing barn where it was first isolated.
2. It may indeed have an undefined specific microecological niche. The organism can grow on and tolerate a wide variety of compounds; however, we have been unable to demonstrate those that are unique to *B. dermatitidis* nor any critical symbiotic organisms.
3. There may indeed be a specific yet undiscovered animal host or carrier. The possible association of the fungus with ground dwelling animals is intriguing. The relationship of thermal and nutritional dimorphism to warm or cold-blooded animals is unknown.
4. *B. dermatitidis* may have a chaotic occurrence in the environment. Its eco-biology is dependent upon a wide variety of variables including its physiology, the physical and chemical processes around it and the presence of competing microorganisms. This fungus may obey the principals of chaos theory [100], and underlying non-linear dynamics may determine its

occurrence as has been described with other ecologies and pathogens.

Some combinations of the above hypotheses may best explain the ecology of this fascinating fungus.

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Contributors

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