



# Ecology of *Cryptococcus neoformans*

Tania C. Sorrell & David H. Ellis

Centre for Infectious Diseases and Microbiology, University of Sydney, Westmead Hospital, New South Wales and The Adelaide Women's and Children's Hospital, South Australia

*Cryptococcus neoformans* is a basidiomycetous, yeast-like fungus which, following inhalation from an environmental source, causes respiratory and neurological disease in humans and animals. Though the ecology of *C. neoformans* is poorly understood, recent observations provide clues about its life-cycle in nature. Two biotypes of *C. neoformans* are recognised, *C. neoformans* var. *neoformans* (serotypes A, D, AD) and *C. neoformans* var. *gattii* (serotypes B, C).

***C. neoformans* var. *neoformans*.** This biotype has been isolated from various sources in nature and is noted for its association with accumulations of avian guano, especially pigeon excreta. It has also been isolated from droppings of caged birds including canaries, parrots and budgerigars. Other environmental isolations have been made from rotting vegetables, wood, dairy products and soil. The pigeon is unlikely to be the major source of cryptococci in nature, since only low concentrations of organisms are found in samples from the beak, crop, feet and rectal swabs [1]. The internal temperature of the bird is 42 °C, which is inhibitory to cryptococcal multiplication; the high concentrations of ammonia in fresh droppings are also inhibitory to growth. In contrast, very high concentrations of the yeast (asexual) form of the organism are found in weathered pigeon droppings, an environment which is unfavourable to the growth of most other microorganisms. The natural environmental niche of *C. neoformans* var. *neoformans* has been predicted to be a plant species, based on the discovery by Ellis and Pfeiffer of certain species of eucalypt as the natural habitat of *C. neoformans* var. *gattii* (see below, [2]). A recent report from Brasil is of great interest in this regard [3]. *C. neoformans* var. *neoformans* was consistently isolated from decaying wood in the hollows of several species of tree in suburban Rio de Janeiro. No specific association with a bird, animal or insect vector could be established.

***C. neoformans* var. *gattii*.** This biotype has a more restricted geographical distribution than *C. neoformans* var. *neoformans*, causing human disease in climates ranging from temperate to tropical in Australia, Papua New Guinea, parts of Africa, India, South-East Asia, Mexico, Brasil, Paraguay and Southern California [4,5]. The first environmental isolation of the organism was made by Ellis and Pfeiffer, in the Barossa Valley of South Australia. These investigators established its specific ecological association with *Eucalyptus camaldulensis*, a species of red gum widely distributed in mainland Australia. Subsequently, another species of red gum, *Eucalyptus*

*tereticornis*, has been confirmed as a natural habitat. High concentrations of *C. neoformans* var. *gattii* have been isolated from single specimens of the related *Eucalyptus rudis* and an unrelated species, *Eucalyptus gomphocephala* (tuart) in the southwest of Western Australia. Three of these species (*E. camaldulensis*, *E. tereticornis*, *E. gomphocephala*) have been exported to several of the countries in which human disease due to *C. neoformans* var. *gattii* has been reported though the association is not exact. Outside of Australia limited isolations of *C. neoformans* var. *gattii* have been made from eucalypts in California and recently, in Apulia, Italy [6], but not in other countries. In Australia, 92% of human isolates and all of those from koalas (a native animal which feeds on the leaves of *E. camaldulensis* and *E. tereticornis*) and host eucalypts, exhibit the same genetic fingerprint (VGI) when identified by random amplification of polymorphic DNA and PCR-fingerprinting [7], consistent with an epidemiological association between mammalian disease and exposure to host eucalypts. The occurrence of disease in countries such as Malaysia which lack the host trees, and our own observation of a distinct genetic type (VGII) in certain locations in Australia suggest that additional environmental niches are yet to be discovered. The life cycle of *C. neoformans* var. *gattii* in association with the trees is unknown. Since both varieties of *C. neoformans* grow well on water-agar containing sterilised eucalyptus leaves, the specificity of the association between *C. neoformans* var. *gattii* and the trees in nature may depend on a eucalypt-associated transport vector. For example, cryptococci may be transported to this niche by animals living within a restricted range (e.g. koalas, which harbour the organism in the web spaces of their claws and move at night between trees), or across greater distances by wind or eucalypt-using birds (consistent with a single molecular type of *C. neoformans* var. *gattii* being distributed widely across Australia). Highest concentrations of the organism (up to 1,000 cfu/g) are found in woody debris in hollows of aged trees, where it is protected from the lethal effect of sunlight and from drying. *C. neoformans* can utilise this woody material, which is rich in polyphenol compounds and lignin, as a substrate for growth, due to its phenol oxidase activity. Following their early observations that cryptococci were isolated from flowers, leaves and air around *E. camaldulensis* trees during the flowering season and that fungal forms morphologically consistent with basidiospores, were present in material from which large numbers of cryptococci were cultured, Ellis and Pfeiffer proposed that the life cycle of the fungus involves basidiospore formation (presumably indicating that the sexual state of the fungus exists in nature) and that dispersal of this potentially infectious propagule occurs during the flowering season [2]. However, cryptococcosis in humans in Australia is not seasonal and the occurrence of high levels of cryptococci in woody debris may indicate a primary association with bark, as has been described for other basidiomycetes [8].

**Dirección para correspondencia:**

Professor Tania C. Sorrell  
Centre for Infectious Diseases and Microbiology,  
Level 3, ICPMR, Westmead Hospital, Darcy Rd,  
Westmead, NSW 2145, AUSTRALIA.  
Fax: (+61-2) 9891 5317  
E-mail: Tanias@cidm.wh.su.edu

**Life cycle of *C. neoformans*.** Sexual reproduction in both varieties of *C. neoformans* has been observed under laboratory conditions. Conjugation between yeast forms of the two mating types ( $MAT\alpha$  and  $MATa$ ) results in the formation of dikaryotic mycelia, basidia, and after meiosis, basidiospores, which germinate into yeasts [9]. The yeast form of *C. neoformans* var. *neoformans* the one which is reportedly present in weathered pigeon droppings. Ellis and Pfeiffer have suggested that basidiospores of *C. neoformans* var. *gattii* occur in host eucalypt material [2]. Until recently, the overwhelming predominance of the  $\alpha$  mating type of both varieties of *C. neoformans* in nature and amongst clinical isolates has been unexplained, since sexual reproduction should result in Mendelian segregation of  $MAT\alpha$  and  $MATa$ , generating equal numbers of progeny of the two mating types (as demonstrated in laboratory crosses). It has been noted that most environmental strains are haploid and heterothallic, though a few may be self fertile, producing basidiospores without apparent mating. The mating type bias has been postulated to be due to increased virulence of the more common  $MAT\alpha$  in clinical specimens, or to the more frequent presence of lethal mutations in  $MATa$  and/or increased sensitivity of this type to unidentified environmental stress.  $MAT\alpha$  is more virulent in mice inoculated with cells of a congenic strain pair of the two mating types [9]. However, a plausible explanation of the mating type bias in nature and natural infection is provided by a recent report of monokaryotic or haploid fruiting (formation of haploid basidia and basidiospores in the vegetative phase) under in vitro conditions of nitrogen starvation and growth on 4% agar medium. This was confined to the  $\alpha$  mating type of both varieties of *C. neoformans* [10]. The phenomenon is consistent with the observations of forms resembling basidiospores in the inhospitable environment of eucalypt debris [2].

**Infectious propagule of *C. neoformans*.** Desiccated yeast cells and basidiospores have been proposed to be the infectious unit of *C. neoformans* since they are sufficiently small (<3  $\mu$ m diameter) to be deposited in lung alveoli following inhalation. Experimentally, culture of yeast cells under conditions of nutrient deprivation and low moisture or in sterilized soil [12] is associated with loss of capsule and a reduction in cell size to 3  $\mu$ m or less. Cryptococci isolated from weathered pigeon droppings exist exclusively in the yeast form and are present predominantly or exclusively as the  $\alpha$ -mating type, consistent with non-sexual replication as haploid yeasts. However, encapsulated yeasts display poor viability under conditions of nutrient deprivation and low moisture [10]. In contrast, basidiospores are resistant to drying, are small (<2  $\mu$ m in diameter), readily become airborne, develop into yeasts on culture and are pathogenic for mice [11]. The recent demonstration of haploid fruiting under laboratory conditions [10] and the observation of basidiospore-like cells in eucalypt debris [2] lends credence to the hypothesis that the basidiospore is the form of *C. neoformans* which is pathogenic for man and animals.

In summary, the ecology of *C. neoformans* remains poorly understood. Recent work has provided exciting avenues for investigation of the natural life-cycle and epidemiology of this organism, which is the commonest cause of human fungal meningitis, and a major cause of disease in patients with AIDS.

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