

Diversity of the *Cryptococcus neoformans*-*Cryptococcus gattii* species complex

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Summary More than 110 years of study of the *Cryptococcus neoformans* and *Cryptococcus gattii* species complex has resulted in an enormous accumulation of fundamental, applied biological and clinical knowledge. Recent developments in our understanding of the diversity within the species complex are presented, emphasizing the intraspecific complexity, which includes species, microspecies, hybrids, serotypes and genotypes. Each of these may have different roles in disease. An overview of obsolete and current names is presented.

Key words *Cryptococcus neoformans*, *Cryptococcus gattii*, Yeast, Taxonomy, Pathogen.

Diversidad del complejo de especies *Cryptococcus neoformans*-*Cryptococcus gattii*

Resumen Más de 110 años de estudio sobre el complejo de especies *Cryptococcus neoformans* y *Cryptococcus gattii* han dado lugar a un gran acúmulo de conocimiento básico, aplicado y clínico. En este artículo se describen los avances recientes en la comprensión de su diversidad, haciendo énfasis en la complejidad intraespecífica que presenta y que llega a englobar especies, microespecies, híbridos, serotipos y genotipos. Cada uno de estos grupos puede jugar un papel diferente en la enfermedad. Se presenta también una visión global de los nombres obsoletos y actuales de estos taxones.

Palabras clave *Cryptococcus neoformans*, *Cryptococcus gattii*, Levaduras, Patógeno, Taxonomía.

Introduction

Studies on *Cryptococcus neoformans* and *Cryptococcus gattii* started in 1894, when Sanfelice first isolated *Saccharomyces neoformans* from peach juice in Italy [139]. In the same year *Saccharomyces hominis* was observed in an infection of a German patient [10,11] and two years later an encapsulated bacilliform yeast, which was named *Saccharomyces subcutaneous tumefaciens*, was isolated from an otherwise healthy man in France [35]. Vuillemin examined several of these cultures, but as the ascospore cha-

racteristics of the genus *Saccharomyces* were not present, he placed these species in the genus *Cryptococcus* [166]. *Cryptococcus hominis* was later described as a synonym to *C. neoformans* [102]. In 1970, an atypical strain of *C. neoformans* was isolated from a leukemic patient and described as *C. neoformans* var. *gattii* [162]. The teleomorph of *C. neoformans* was discovered when two serotype D strains were crossed and was named *Filobasidiella neoformans* [74]. However, when serotype B and C strains were crossed, a teleomorph that clearly differed from *F. neoformans* was observed. This resulted in the description of *Filobasidiella bacillisporea* [76]. In addition, several physiological assays showed that *C. neoformans* serotype B and C strains differed from *C. neoformans* serotype A and D isolates, and the name *Cryptococcus bacillisporeus* was proposed for serotype B and C isolates [81]. Although previous mating experiments with *C. neoformans* var. *gattii* had not been successful [81], viable basidiospores were produced when strains of *C. neoformans* var. *gattii* were crossed with isolates of *F. bacillisporea* and *F. neoformans* [141]. Furthermore, mating of isolates of *F. bacillisporea* with *F. neoformans* resulted in the formation of viable basidiospores [80,141]. Kwon-Chung et al. [80] therefore proposed to treat the two species as varieties of *C. neoformans*, namely *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii*. In 1999, based on phenotypic and genotypic data Franzot et al. [51] proposed to install a third variety, namely *C. neoformans* var. *grubii*, corresponding to se-

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rotype A. Despite the results of earlier mating experiments, *C. gattii* has recently been described as a separate species, because of the increasing amount of data suggesting the distinctiveness of *C. neoformans* and *C. neoformans* var. *gattii* [82]. Table 1 presents an overview of names in old and current use within the species complex.

Currently, two species are recognized within the *C. neoformans* - *C. gattii* species complex. These species are the asexual yeast *C. neoformans* (Sanfelice) Vuillemin [166], with teleomorph *F. neoformans* Kwon-Chung [74] and the asexual yeast *C. gattii* (Vanbreuseghem and Takashio) Kwon-Chung et al. [82] with *F. bacillispora* Kwon-Chung as teleomorph [76].

Both *C. neoformans* and *C. gattii* belong to the *Filobasidiella*-clade of the *Tremellales* (Basidiomycota, Agaricomycotina, Tremellomycetes) [47,63,143]. *Cryptococcus amylolentus*, *Tsuchiyaea wingfieldii* and *Filobasidiella depauperata*, species which are closely related to *C. neoformans* and *C. gattii* [47,48,60,83,113,143], have been isolated either from insect frass or from dead insects (www.cbs.knaw.nl/yeast/BioloMICS.aspx).

Infection and virulence

A pulmonary infection with *C. neoformans* or *C. gattii* most likely starts with inhalation of infectious particles, which have not been identified with certainty, although basidiospores seem to be the most likely candidate [20]. Yeast cells are too large to penetrate into the alveoli [62] where infection starts and desiccated yeast cells display poor viability [136, 169]. Inhaled basidiospores may cause infection in mice and are more effective than yeast cells in causing cryptococcosis [152]. Interestingly, humans without a history of cryptococcal disease have antibodies against *C. neoformans* [30]. Furthermore, the majority of children have been exposed to *C. neoformans* before they reach the age of five [56]. These results show that humans frequently come into contact with *C. neoformans*. In immunocompetent individuals *C. neoformans* infection is either cleared or remains dormant [55]. In immunocompromised individuals, however, *C. neoformans* can disseminate to other organs. Infection of the central nervous system may result in meningoencephalitis, a condition that is fatal if left untreated. Contrary to *C. neoformans*, *C. gattii* primarily infects otherwise healthy individuals [31,111,112,135,147], but serotype C isolates of *C. gattii* were found to be implicated in HIV-associated infections in California, Botswana and Malawi [27,97]. The reported incubation period for *C. gattii* serotype B, genotype AFLP/6/VGII of up to six or seven months [104] or as short as two months [96], is consistent with infection by a primary pathogen. Although both species infect the central nervous system, *C. gattii* appears to invade the brain parenchyma more commonly than *C. neoformans*. Furthermore, in *C. gattii* infected patients, pulmonary infections are more likely and pulmonary mass-like lesions are more common than in *C. neoformans* infected patients [111,147].

Although *C. neoformans* is a human pathogen, the human host is probably an accidental encounter and not its primary niche. Infection of macrophages and amoebae by *C. neoformans* is similar, and it was therefore postulated that mammalian virulence factors of *C. neoformans* evolved as a defense mechanism to environmental predators [148,149]. There are several virulence factors that contribute to the virulence of *C. neoformans* in mammals. Obviously, the ability to grow at 37 °C is essential. *C. neoformans* and *C. gattii* are the only *Tremellales* that can

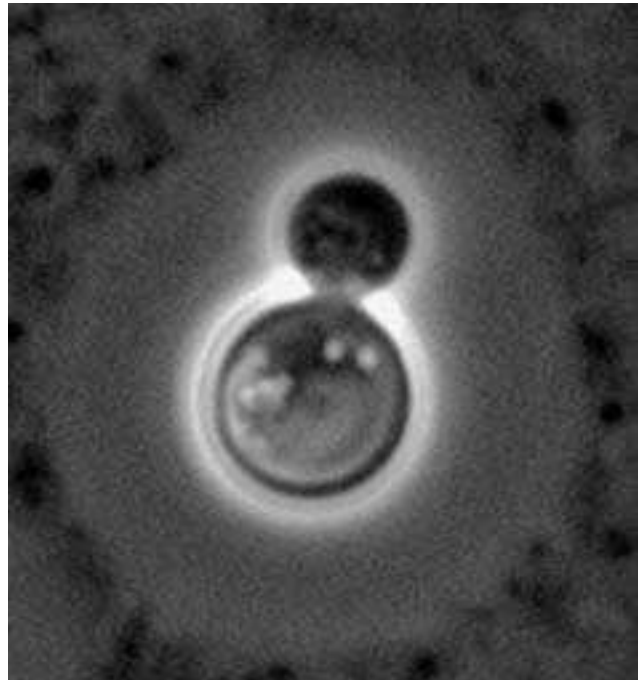


Figure 1. India ink stained capsule around budding yeast cell of *Cryptococcus gattii* CBS 883 after two days at room temperature on Littmann agar.



Figure 2. Melanin (JEC 21, right) and non-melanin (JEC 20, left) forming colonies of *C. neoformans* serotype D on L-DOPA medium after 5 days at 25 °C.

grow optimally at temperatures above 30 °C [86,129] and temperature sensitive mutants of *C. neoformans* are attenuated in virulence [128]. Another important virulence factor is the production of a large polysaccharide capsule (Fig. 1). Acapsular mutants are avirulent in mouse models [22-25]. Capsule size increases during infection [46] and can be induced *in vitro* by high CO₂ concentration [59], iron deprivation [163], and serum [178]. Although some properties of the capsule enable the host to clear *C. neoformans* more effectively, others protect *C. neoformans* against host defenses. Overall, the effect of the capsule is beneficial: encapsulated cryptococcal cells are not phagocytized or killed by neutrophils, monocytes or macrophages to the same degree as acapsular cells [9]. The production of melanin is another important virulence factor (Fig. 2). Melanin synthesis is catalyzed by a laccase and may occur when phenolic compounds, e.g. catecholamines, are present [127,130,172,173]. Dopamine, a catecholamine which is present in the brain, is a substrate for melanin synthesis [10,172]. The ability of *C. neoformans* and *C. gattii* to form melanin has been used to develop

diagnostic selective media, such as L-DOPA or nor-epinephrine media. It is important to note that also non-*neoformans* cryptococci are able to form melanin, such as *C. podzolicus* and *Cryptotrichosporon anacardii* [124,129]. In *C. neoformans* and *C. gattii*, melanin is synthesized during infection [122,134] and mutants that do not produce melanin are less virulent [133,138]. Melanin protects *C. neoformans* from oxidative damage by scavenging host-produced anti-oxidants [9,167]. In addition, laccase decreases the amount of hydroxyl radicals directly, thereby protecting *C. neoformans* [101]. Given the notion that *C. neoformans* may represent a secondary (or dormant) pathogen contrary to *C. gattii* (or at least some of the genotypes occurring in this species) it would be particularly interesting to study the main virulence traits in both in order to understand the molecular basis of these differences in pathogenicity. This is not only important from a clinical point of view, but may also largely contribute to our understanding of speciation within this complex of important human and animal pathogens. Recently, in addition to vertebrates, such as the mouse, rat and rabbit, a number of invertebrate model organisms have been explored for their potential contributions to our understanding of cryptococcal pathogenesis [103]. Experiments with *Caenorhabditis elegans*, a widely used model organisms in molecular biology, showed that capsule and G- α protein-cAMP-protein kinase A (PKA) played a role in cryptococcal virulence [116]. Also *Drosophila melanogaster* and *Galloria mellonella* have been used for cryptococcal virulence assays. Because each of these model organisms have their advantages and disadvantages [103], it may well be that an integrated approach using multiple vertebrate and invertebrate hosts is needed to understand the full extent of cryptococcal pathogenicity.

Epidemiology and ecology

C. neoformans and *C. gattii* can be distinguished by serotype (A, D and AD for the former, B and C for the latter) [80,82], karyotype [6,171], RAPD patterns [6,40,137], PCR fingerprints [110], RFLP patterns [89,108], AFLP fingerprint [7] and DNA sequencing [5,15,28,37,38,69,151,175,176]. Most of these methods have shown that two genotypic groups can be recognized within *C. neoformans* and these groups have been described as separate varieties, namely var. *grubii* (serotype A) and var. *neoformans* (serotype D) [51] (see Table 1). However, although several molecular methods distinguish four genotypic groups within *C. gattii* [5,15,28,37,40,53,89,108,137], these groups have not yet been interpreted as distinct taxa (see Table 1). Interestingly, *C. gattii* serotypes B and C do not correspond to a specific group, but appear to occur in all genotypic groups [108].

C. neoformans and *C. gattii* differ in epidemiology and in their ecological niche. *C. neoformans* var. *grubii* occurs throughout the world and causes 99% of the cryptococcal infections in HIV-infected patients [111]. In Europe this variety occurred in 51% of the isolates analyzed in a 30-month survey [165]. *C. neoformans* var. *neoformans* occurs worldwide as well, but appears to be more common in Europe, where 30% of the reported infections are caused by this variety [39,79,158,159,165]. In France, it was found that infection by this variety occurred more frequently among patients from certain geographical areas, among patients receiving corticoid steroid treatment, and in patients with skin lesions [39]. In contrast, the risk of being infected was smaller among female patients, those originating from Africa, and for patients suffering from

Table 1. Names in current and old use in the *C. neoformans* / *C. gattii* species complex and with reference to the different typing nomenclatures in current use.

1. *Cryptococcus neoformans*, type strain CBS 132¹
Teleomorph *Filobasidiella neoformans*, holotype BPI 71843 (= CBS 6885 [serotype D, AFLP 2] × CBS 6886 [serotype D, AFLP 2]).
– *Cryptococcus neoformans* var. *neoformans*, type strain CBS 132¹ = serotype D = AFLP genotype 2 = genotype VNIV [reference strain WM629 = CBS 10079]. Synonyms for which type is available: *Saccharomyces neoformans*, type CBS 132²; *Torula nasalis*, type strain CBS 882.
– *Cryptococcus neoformans* var. *grubii*, type strain CBS 8710 = serotype A = AFLP genotype 1/1A/1B = genotype VNI/VNII [reference strains WM148/WM626 = CBS 10085/10083]. Synonyms for which type or authentic strain is available: *Candida psicrophylicus*³, type CBS 996; *Saccharomyces hominis*⁴, authentic strain CBS 879.
2. Intervarietal *grubii* × *neoformans* hybrids
– Serotype AD Hybrids = AFLP genotype 3¹ = genotype VNIII [reference strain WM 628 = CBS 10080]. Note that CBS 132, the type strain of *C. neoformans* variety *neoformans* is a serotype AD hybrid.
3. *Cryptococcus gattii*³, type strain CBS 6289 = RV 20186.
Teleomorph *Filobasidiella bacillispora*, holotype BPI 71855 (= CBS 6956 [serotype B, AFLP 6] × CBS 6993 [serotype C, AFLP 5])⁴
No infraspecific taxa are described, but four different genotypes do occur.
– AFLP genotype 4 = serotype B (or C)⁵ = genotype VGI [reference strain WM179 = CBS 10078].
Synonyms for which type or authentic strains are available: *Cryptococcus neoformans* var. *gattii*, type strain CBS 6289; *Candida honduriansis*³, syntype strain CBS 883; *Torulopsis neoformans* var. *sheppeii*, type strain CBS 919; *Cryptococcus neoformans* var. *shanghaiensis*, type strain CBS 7229; *Saccharomyces subcutaneous tumefaciens*⁵, authentic strain CBS 1622.
– AFLP genotype 5 = serotype B or C = genotype VGIII [reference strain WM161 = CBS 10081]. Synonym for which a type strain is available: *Cryptococcus bacillisporus*, type strain CBS 6955.
– AFLP genotype 6 = serotype B (or C)⁶ = genotype VGII [reference strain WM 178 = CBS 10082].
– AFLP genotype 7 = serotype B or C = genotype VGIV [reference strain WM779 = CBS 10101].
4. Interspecific *gattii* × *neoformans* hybrids
– Serotype BD hybrid = AFLP genotype 8 [reference strain CBS 10488].
– Serotype AB hybrid = AFLP genotype 9 [reference strain CBS 10496].

¹ The type strain of *Cryptococcus* (= *Saccharomyces*) *neoformans* CBS 132 is a serotype AD hybrid. When both serotypes A and D will be recognized as separate species, this type strain may need to be replaced in order to establish nomenclatural stability.

² These names have priority at the species level.

³ *Cryptococcus gattii* is conserved against *Cryptococcus honduriansis*.

⁴ These two strains belong to different genotypes within *C. gattii* as follows: CBS 6956 (NIH 444) is AFLP6/VGII, whereas CBS 6993 is AFLP5/VGIII. Given the many genomic differences between these two genotypes, it may be that they represent different infraspecific taxa or even microspecies.

⁵ This name is an illegitimate trinomial.

⁶ Although serotype C has been reported to occur in these genotypes, this could not be confirmed by the present authors. This, however, may be due to the different strain collections used in the various studies.

meningeal cryptococcosis [39]. In Europe, 30% of the isolates were found to represent this variety, and in Italy it was most frequently isolated in the Northern part of the country [165]. In contrast to *C. neoformans*, *C. gattii* mainly infects otherwise healthy individuals [31,111,135,147] and occurs predominantly in subtropical areas [79,100,108]. *C. gattii* has, however, also been isolated in Europe in areas with a temperate or Mediterranean climate [3,34,35,114,164] and it is also known from temperate climate zones in Colombia [45]. Furthermore, *C. gattii* is responsible for the ongoing outbreak of cryptococcosis on Vancouver Island, Canada [65,72,150], and has recently been detected in other areas in the Pacific Northwest, USA [105].

C. neoformans is often isolated from avian excreta, mainly from pigeon excreta [20,44]. In addition, it has been isolated from soil [43] and decaying wood [91]. Zoonotic transmission of *C. neoformans* from bird's excreta to immunocompromised or immunocompetent humans has been demonstrated in a few cases [88,121] or has been seriously considered in others [145].

C. gattii has been isolated from several tree species since the initial finding of *C. gattii* on *Eucalyptus camaldulensis* [16,41,42,45,49,54,57,72,73,90,92,131,132]. Recently, however, it has been suggested that soil may in fact be the principal reservoir for this species [71]. In a detailed analysis of Colombian isolates it was observed that climatic conditions were related to the distribution of both *C. neoformans* var. *grubii* and serotypes B and C of *C. gattii*. It seems likely therefore that differential tolerances of the genotypes/serotypes to environmental conditions, such as climate, may effect their geographic and ecologic distributions [58].

Reproduction

C. neoformans and *C. gattii* are usually haploid yeasts that predominantly reproduce asexually, i.e. by budding. However, they also possess a bipolar mating system, with mating-types α and α [74,75]. The mating-type (*MAT*) locus is the region of a fungal genome that regulates the sexual cycle and which is different between cells of opposite mating-type. Mating may occur if cells of opposite mating-types meet [74-76]. *C. neoformans* possesses a single *MAT* locus, which is unusually large, i.e. more than 100 kb for both *C. neoformans* and *C. gattii* [52,53,94]. It encodes more than twenty genes, including homeodomain genes which establish cell type identity, genes involved in pheromone production and sensing, components of a MAP kinase cascade, essential genes, and genes which do not seem to have a function in mating [52,94,115]. Evidence suggests that the ancestor of *C. neoformans* had two unlinked sex-determining regions, which expanded by acquisition of genes of related function. A chromosomal translocation fused the two regions, which resulted in a tripolar intermediate mating system that collapsed into a bipolar system. In this bipolar system inversions, that suppressed recombination, occurred, which resulted in the currently known *MAT* loci [52].

MAT α cells produce *MF α* pheromone, e.g. in response to nitrogen starvation, and in response to this pheromone *MAT α* cells form a conjugation tube [26,168]. In response to *MF α* pheromone, which is induced by starvation conditions as well as by the presence of *MAT α* cells [144], the *MAT α* cells dramatically enlarge to form large swollen cells that can fuse with the conjugation tubes of the *MAT α* cells [36]. McClelland et al. [107] hypothesized about the cascade of events following conjugation tube formation by the *MAT α* cell. The *MAT α* nucleus divides and migrates

into the conjugation tube. Simultaneously, the *MAT α* nucleus divides and the *MAT α* cell initiates hyphal formation. Nuclei from both mating-types then migrate into the hyphae. The now dikaryotic hyphae are linked by fused clamp connections. A basidium is formed on the tip of hyphae and subsequently karyogamy and meiosis occur within the basidium [75]. The four resulting nuclei remain in the basidium and repeated post-meiotic mitosis generates four long chains of spores [75,77]. A single spore chain may contain parental spore types as well as recombinants, indicating that the nuclei in the basidium are randomly distributed and that mitosis of these nuclei occurs randomly prior to spore formation [77].

C. neoformans cells may also reproduce by haploid fruiting, which occurs in response to nitrogen starvation and/or desiccation [170]. Haploid fruiting resembles mating, but there are some differences. Mating involves partners of opposite mating-types, whereas haploid fruiting occurs in strains of the same mating-type [170]. In addition, nuclear fusion occurs early during haploid fruiting [95], but late during mating [75]. Furthermore, clamp connections of filaments produced during mating are fused, whereas clamp connections produced during fruiting are unfused [170]. During haploid fruiting basidia with viable basidiospores are formed, albeit at a lower frequency than in a regular *MAT α* \times *MAT α* cross [170]. Although haploid fruiting has first been described in *MAT α* isolates of all serotypes [170], it has also been observed in a few *MAT α* isolates [161]. Interestingly, one of the environmental *C. gattii* isolates from Vancouver Island is a diploid homozygous *MAT α* strain [53], which may have been generated by aberrant 'haploid fruiting', i.e. same sex mating [95].

Several purposes have been suggested for haploid fruiting, or more specifically for the resulting filamentation. Haploid fruiting may increase the chance of finding a mating partner [67,168]. An indication for this is the stimulation of haploid fruiting of *MAT α* cells in response to *MF α* pheromone [144]. Haploid fruiting may also increase the foraging capacity under low nutrient conditions, as is indicated by the observation that haploid fruiting of *MAT α* cells is enhanced by overexpression of *MF α* pheromone genes, which are induced during starvation conditions [144]. Recently, a phenomenon called same-sex mating, i.e. mating between two non-isogenic *MAT α* cells, has been described [95]. The isolation of serotype A *MAT α* -serotype D *MAT α* environmental isolates [97] suggests that same-sex mating occurs in the environment.

Although mating of *C. neoformans* or *C. gattii* was shown to occur under laboratory conditions [74, 76], it has never been found in the environment. In addition, past studies have found evidence for clonal population structure [12,13,29,50]. However, when *C. neoformans* var. *grubii* and var. *neoformans* were studied separately the null hypothesis of recombination could no longer be rejected [157]. In addition, analysis of the *LAC1* and *URA5* genes for AD hybrid isolates showed that recombination occurred within each variety [175]. Furthermore, recombination may occur within subpopulations of var. *grubii* [97,99] and within populations of var. *neoformans* [97]. In addition, recombination was found in subpopulations of *C. gattii* AFLP6 [18]. In summary, *C. neoformans* and *C. gattii* reproduce through diverse sexual and asexual strategies, in which both recombination and clonal dispersal do occur [17].

The majority of environmental and clinical isolates belongs to *MAT α* . [1,18,19,21,29,45,54,61,64,66,68,78,97,99,100,106,123,125,126,132,140,142,154,160,165,177]. However, *C. gattii* *MAT α* and *MAT α* isolates have been

found in an 1:1 ratio and *MATa* isolates sometimes even outnumbered the *MAT α* isolates [45,61]. Unequal inheritance of the *MAT* locus could provide an explanation for the excess of *MAT α* isolates. However, in laboratory crosses *MATa* and *MAT α* isolates were either obtained in an 1:1 ratio [70,75,77,80,99,118,161,170] or an excess of *MATa* progeny was obtained [80,161]. When haploid fruiting in *MAT α* isolates was first described [170] this phenomenon was thought to explain the high number of *MAT α* isolates in environmental and clinical isolates. However, Tschärke et al. [161] showed that *MATa* isolates are also capable of haploid fruiting. In addition, the poor haploid fruiting capability of *C. neoformans* var. *grubii* isolates, which are responsible for the majority of cryptococcal infections [111,161,170], indicated that haploid fruiting does not provide an explanation for the high number of *MAT α* .

It has also been suggested that *MAT α* isolates might be more virulent than those belonging to *MATa*. Several approaches have been used to test this hypothesis. Congenic pairs of both serotype A and D were created and although virulence was associated with *MAT α* in the serotype D congenic pair [84], no clear association between virulence and mating-type was found when progeny of a serotype D mating was compared [85]. Furthermore, the serotype A congenic pair did not differ in virulence [118]. Interestingly, during *C. neoformans* var. *grubii* *MATa* and *MAT α* coinfection *MAT α* cells out-competed *MATa* in entry to the central nervous system. Nielsen et al. [117] and Barchiesi et al. [2] found that the presence of a serotype A-*MAT α* allele is associated with virulence. Finally, virulence studies carried out using serotype D congenic pairs of different genetic background showed that the genetic background plays a significant role in determining the effect of mating-type on virulence [119].

Hybrids

Occasionally diploid or aneuploid isolates, such as AD, BD or AB hybrids, are found [4,8,14,32,33,53,64,79,97,100,109,120,140,153,154,156,158-160,165, M. Bovers, F. Hagen & T. Boekhout, unpublished observations], although the diploid phase of *C. neoformans* and *C. gattii* is normally associated with the filamentous sexual stage rather than the yeast stage. Hybrids usually possess both mating-type loci which indicates that they most likely result from mating between isolates of opposite mating-types [8,32,93]. Hybrids can result either from pre-meiotic sporulation or from fusion of haploid meiotic nuclei followed by the packing of a diploid nucleus in a basidiospore [146]. Indeed, Kwon-Chung [75] has observed atypical basidia producing two normal spores and one spore which was twice as large and may have contained a diploid nucleus. These observations are consistent with the formation of hybrids by fusion of haploid meiotic nuclei.

Genetic and molecular studies performed on AD hybrid isolates showed that these were formed multiple times [174,177] and AD as well as BD hybrids have been generated in the lab [33,87,93,146,155]. When serotype A and serotype D strains were mated, 24% to 70% of the progeny were diploid or aneuploid [33,87,155]. In addition, mating of a serotype B with a serotype D strain resulted in 50% diploid or aneuploid progeny [87]. These results show that AD and BD hybrids can be generated quite easily. In some areas of Europe, 16 to 30% of the clinical isolates were found to be AD hybrids [32,79,165], with Spain, Greece and Portugal showing the highest incidence, namely 45%, 48% and 50%, respectively [165]. These observations indicate that in these areas hybrids are responsible for a significant part of the cryptococcal infections.

Serotype AD hybrids may contain both *MATa* and *MAT α* alleles for both serotypes A and D, and, hence, both AaD α and A α Da genotypes occur [33,97, M. Bovers & T. Boekhout, unpublished observations]. Recently it was proposed that the AaD α genotype may have originated from a mating population in Botswana, which, due to better fitness characteristics could disseminate globally [98].

Conclusions and perspective

C. neoformans and its sibling species *C. gattii* form a species complex that has been studied from many different perspectives. The results suggest that these two species are distinct. Accumulating evidence, however, suggests that the varieties *grubii* and *neoformans*, and probably the four genotypes in *C. gattii* may also represent separate microspecies. Further molecular, genetic and ecological investigations are needed to confirm this hypothesis. The existence of both intervarietal (serotype AD) and interspecific (serotype BD and AB) hybrids, which are diploid or aneuploid, indicate that species boundaries may be maintained by postzygotic isolation mechanisms. The continued sampling of clinical and environmental isolates, together with adequate genotyping, may further strengthen the role of each genotype in the various diseases caused by representatives of the species complex, as well as the various patient categories in which cryptococcosis occurs.

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