Uniparental sexual reproduction following cell-cell fusion of opposite mating-type partners

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Abstract

Some animal species require an opposite-sex partner for their sexual development but discard the partner's genome before gamete formation, generating hemi-clonal progeny in a process called hybridogenesis. In this study, we discovered hybridogenesis-like reproduction in a basidiomycete fungus, *Cryptococcus neoformans*. *C. neoformans* has two mating types, *MAT***a** and *MAT*α, which fuse to produce a dikaryotic zygote that completes a sexual cycle producing recombinant meiotic progeny. Here, we discovered exclusive uniparental inheritance of nuclear genetic material in a fraction of the F1 progeny produced during bisexual reproduction of two opposite mating-type partners. By analyzing strains expressing fluorescent reporter proteins, we observed that dikaryotic hyphae were produced, but only one parental nuclei was found in the terminal basidium where sporulation occurs. Whole-genome sequencing revealed the nuclear genome of the progeny was identical with one or the other parental genome, whereas the mitochondrial genome was always inherited from the *MAT***a** parent. Uniparental sporulation was also observed in natural isolate crosses occurring in concert with biparental sporulation. The meiotic recombinase Dmc1 was found to be critical for uniparental reproduction. These findings reveal an unusual mode of eukaryotic microbial unisexual reproduction that shares features with hybridogenesis in animals.

Introduction

Most organisms in nature undergo sexual reproduction between two partners of the opposite sex to produce progeny. In most cases, the diploid zygote receives one copy of the genetic material from each parent following fusion of the two haploid gametes. To produce these haploid gametes, the diploid cell of the organism undergoes meiosis, which involves recombination between the two parental genomes, generating recombinant progeny. Recombination confers benefits by bringing together beneficial mutations and segregating away deleterious ones (Dimijian 2005, Meirmans 2009). In contrast, some organisms undergo variant forms of sexual reproduction, including parthenogenesis, gynogenesis, androgenesis, and hybridogenesis, and, in doing so, produce clonal or hemi-clonal progeny (Neaves and Baumann 2011, Avise 2015).

In parthenogenesis, a female produces clonal progeny from its eggs without any contribution from a male partner (Hörandl 2009, Avise 2015). Gynogenesis and androgenesis occur when the fusion of an egg with a sperm induces cell division to produce clonal female or male zygotes, respectively (Lehtonen et al. 2013). During hybridogenesis, an egg from one species fuses with the sperm from another species to generate a hybrid diploid zygote (Lavanchy and Schwander 2019). However, one of the parental genomes is excluded during development, in a process termed genome exclusion that occurs before gametogenesis. The remaining parental genome undergoes replication followed by meiosis to produce an egg or a sperm. The sperm or egg then fuses with an opposite-sex gamete to generate a hemiclonal progeny. Because only one parent contributes genetic material to the progeny, but both parents are physically required, this phenomenon has been termed sexual parasitism (Umphrey 2006, Lehtonen et al. 2013). While most of the reported cases of hybridogenesis are from female populations, recent reports suggest that it may also occur in male populations of some species (Dolezalkova et al. 2016, Schwander and Oldroyd 2016). Currently hybridogenesis has only been observed in the animal kingdom in some species of frogs, fishes, and snakes. Plants also exhibit parthenogenesis (aka apomixis), along with gynogenesis and androgenesis (Lehtonen et al. 2013, Mirzaghaderi and Horandl 2016).

Unlike animals, most fungi do not have sex chromosomes; instead, cell-type identity is defined by the mating-type (*MAT*) locus (Heitman et al. 2013, Heitman 2015). While many fungi are heterothallic and undergo sexual reproduction involving two partners of compatible mating-types, other fungi are homothallic and can undergo sexual production during solo culture in the absence of a mating partner. One class of homothallic fungi undergoes

unisexual reproduction, during which cells of a single mating type undergo sexual reproduction to produce clonal progeny, similar to parthenogenesis (Lee et al. 2010, Heitman 2015). Gynogenesis and hybridogenesis have not been identified in the fungal kingdom thus far.

Cryptococcus neoformans is a basidiomycete human fungal pathogen that exists as either one of two mating types, *MAT***a** or *MAT*α (Sun et al. 2019a). During sexual reproduction, two yeast cells of opposite mating type interact and undergo cell-cell fusion (Sun et al. 2019b). The resulting dikaryotic zygote then undergoes a morphological transition and develops into hyphae whose termini mature to form basidia. In the basidium, the two parental nuclei fuse (karyogamy), and the resulting diploid nucleus undergoes meiosis to produce four daughter nuclei (Kwon-Chung 1976, Idnurm 2010, Sun et al. 2019b, Zhao et al. 2019). These four haploid nuclei repeatedly divide via mitosis and bud from the surface of the basidium to produce four long spore chains. Interestingly, apart from this canonical heterothallic sexual reproduction, a closely related species, *C. deneoformans* can undergo unisexual reproduction (Lin et al. 2005).

In a previous study, we generated a genome-shuffled strain of *C. neoformans*, VYD135α, by using the CRISPR-Cas9 system targeting centromeric transposons in the lab strain H99α. This led to multiple centromere-mediated chromosome arm exchanges in strain VYD135α when compared to strain H99α, without any detectable changes in gene content in the two genomes (Yadav et al. 2020). Additionally, strain VYD135α exhibits severe sporulation defects when mated with strain KN99**a** (which is congenic with strain H99α but has the opposite mating type) likely due to the extensive chromosomal rearrangements introduced into the VYD135α strain. In this study, we show that the genome-shuffle strain VYD135α can in fact produce spores in crosses with *MAT***a** *C. neoformans* strains after prolonged mating. Analysis of these spores reveals that the products from an individual basidium contain genetic material derived from only one of the two parents. Whole-genome sequencing of the progeny revealed an absence of recombination between the two parental genomes. The mitochondria in these progeny were found to always be inherited from the *MAT***a** parent, consistent with known mitochondrial uniparental inheritance (UPI) patterns in *C. neoformans* (Sun et al. 2020). Using strains with differentially fluorescently-labeled nuclei, we discovered that in a few hyphal branches as well as basidia, only one of the parental nuclei was present and produced spores. We also observed the occurrence of such uniparental inheritance in wild-type and natural isolate crosses. Furthermore, we found that

the meiotic recombinase Dmc1 plays a central role during partner-stimulated uniparental sexual reproduction of *C. neoformans*. Overall, this process of uniparental sexual reproduction of *C. neoformans* exhibits striking parallels with hybridogenesis in animals.

Results

Chromosomal translocation strain exhibits unusual sexual reproduction

Previously, we generated a strain (VYD135α) with multiple centromere-mediated chromosome translocations compared to the wild-type parental isolate H99α (Yadav et al. 2020). Co-incubation of the wild-type strain KN99**a** with the genome-shuffle strain VYD135α resulted in hyphal development and basidia production, but no spores were observed during a standard two-week incubation. However, when sporulation was assessed at later time points in the VYD135α x KN99**a** cross, we observed a limited number of sporulating basidia (16/1201=1.3%) after five weeks of mating compared to a much greater level sporulation in the wild-type H99α x KN99**a** cross (524/599 = 88%) (Figure S1A-B). To analyze this sexual process in detail, spores from individual basidia were dissected and germinated to yield viable F1 progeny. As expected, genotyping of the mating-type locus in the H99α x KN99**a** progeny revealed the presence of both mating types in spores derived from each basidium. On the other hand, the same analysis for VYD135α x KN99**a** revealed that all germinating progeny from each individual basidia possessed either only the *MAT*α or the *MAT***a** locus (Table 1). PCR assays also revealed that the mitochondria in all of these progeny were inherited from the *MAT***a** parent, in accord with known uniparental mitochondrial genome inheritance. These results provide evidence for the inheritance of only one of the parental nuclei in the VYD135α x KN99**a** F1 progeny. Furthermore, the presence of mitochondria from the *MAT***a** parent suggested that these progeny were not the products of unisexual reproduction.

Fluorescence microscopy reveals uniparental reproduction after mating

Next, we tested whether the uniparental inheritance detected at the *MAT* locus also applies to the entire nuclear genome. To address this, we established a fluorescence-based assay where the nucleus of strains H99α and VYD135α was labeled with GFP-H4, whereas the KN99**a** nucleus was marked with mCherry-H4. In a wild-type cross (H99α x KN99**a**), the nuclei in the hyphae as well as in the spores are yellow to orange because both nuclei are in a

common cytoplasm and thus incorporate both the GFP- and the mCherry-tagged histone H4 proteins (Figure S2). We hypothesized that in the cases of uniparental sporulation, only one of the nuclei would reach the terminal basidium and thus would harbor only one fluorescent nuclear color signal (Figure S2A).

After establishing this fluorescent tagging system using wild-type strains, H99α x KN99**a** and shuffle-strain VYD135α x KN99**a** crosses with fluorescently labeled strains were examined. In the wild-type cross, most of the basidia formed robust spore chains with both fluorescent colors observed in them while a small population (~1%) of basidia exhibited spore chains with only one color, representing uniparental reproduction (Figure 1A and S3A). On the other hand, the majority of the basidia population in the shuffle-strain VYD135α x KN99**a** cross did not exhibit sporulation, and the two parental nuclei appeared fused but undivided (Figure 1B and S3B). A few basidia (~1%) bore spore chains with only one fluorescent color, marking uniparental sporulation events. While the uniparental sporulation events in the H99α x KN99**a** cross were a small fraction (~1%) compared to those resulting from biparental sporulation, the uniparental basidia accounted for all of the sporulating basidia in the VYD135α x KN99**a** cross.

Uniparental reproduction stems from hyphal branches

Fluorescence microscopy revealed that only one of the two parental nuclei is present in a small proportion of the basidia, which results in uniparental meiosis and sporulation. Based on this finding, we hypothesized that the basidia with only one parental nucleus might arise due to nuclear segregation events during hyphal branching. To gain further insight into this process, the nuclear distribution pattern along the sporulating hyphae was studied. As expected, imaging of long hyphae in the wild-type cross revealed the presence of pairs of nuclei with both fluorescent markers along the length of the majority of hyphae (Figure 2A). In contrast, tracking of hyphae from basidia with spore chains in the genome-shuffle strain VYD135α x KN99**a** cross revealed hyphal branches with only one parental nucleus, which were preceded by a hypha with both parental nuclei (Figure 2B).

These results suggest that hyphal branching may facilitate the separation of one parental nucleus from the main hyphae harboring both parental nuclei. As a result, one of the parental genomes is excluded at a step before meiosis and the generation of F1 progeny, similar to the process of genome exclusion observed in hybridogenesis. Nuclear segregation can be followed by endoreplication occuring in these hyphal branches or in the basidia to

produce a diploid nucleus that then ultimately undergoes meiosis and produces uniparental progeny.

Uniparental reproduction also occurs in natural isolates

After establishing the uniparental sporulation in lab strains, we attempted to determine whether such events also occur with natural isolates. For this purpose, we selected two wild-type natural isolates, Bt63**a** and IUM96-2828**a** (referred to as IUM96**a** hereafter) (Keller et al. 2003, Litvintseva et al. 2003, Desjardins et al. 2017). IUM96**a** belongs to the same lineage as H99α/KN99**a** (VNI) and exhibits approximately 0.1% genome divergence from the H99α reference genome. Bt63**a** belongs to a different lineage of the *C. neoformans* species (VNBI) and exhibits ~0.5% genetic divergence from the H99α/KN99**a** genome. Both the Bt63**a** and the IUM96**a** genomes exhibit one reciprocal chromosome translocation with H99α and, as a result, share a total of ten chromosome-level changes with the genome-shuffle strain VYD135α (Figure 3A).

During mating, the H99α x Bt63**a** cross rapidly (within a week) produced robust sporulation from most of the basidia observed. The VYD135α x Bt63**a** cross underwent a low frequency of sporulation (12 spore-producing basidia/840 basidia=1.3%) in 2 to 3 weeks (Figure S1C-D). Dissection of spores from the H99α x Bt63**a** cross revealed a low germination rate (average of 25%) with two of the basidia showing no spore germination at all (Table S1). This result is consistent with previous results and the low germination rate could be explained by the genetic divergence between the two strains (Morrow et al. 2012). Genotyping of germinated spores from the H99α x Bt63**a** cross revealed both *MAT***a** and *MAT*α progeny from individual basidia, with almost 75% of the meiotic events generating progeny that were aneuploid for the *MAT* locus. For the VYD135α x Bt63**a** cross, spores from 15/20 basidia germinated and displayed higher germination rates than the H99α x Bt63**a** cross (Table S1). Interestingly, all germinated progeny harbored only the *MAT*α mating-type whereas the mitochondria were inherited from the *MAT***a** parent. These results suggest uniparental sporulation also occurs with Bt63**a** and accounts for high germination rates of progeny from the VYD135α x Bt63**a** cross. The occurrence of uniparental sporulation was also identified using the fluorescence-based assay with crosses between the GFP-H4 tagged VDY135α and mCherry-H4 tagged Bt63**a** strains (Figure S4).

Mating assays with strain IUM96**a** also revealed a low level of sporulation (19/842=2.2%) with VYD135α but a high sporulation rate with H99α (91%) (Figure S1E-F).

Analysis of progeny from crosses involving IUM96**a** revealed a similar pattern to what was observed with crosses involving KN99**a**. The progeny from H99α x IUM96**a** inherited both *MAT***a** and *MAT*α in each basidium, whereas VYD135α x IUM96**a** progeny from each basidium inherited exclusively either *MAT***a** or *MAT*α (Table S2). Interestingly, we also observed co-incident uniparental *MAT* inheritance and with a high germination rates in basidia 7, 8, and 9 from the H99α x IUM96**a** cross as well (Table S2). Taken together, these results suggest that this unusual mode of sexual reproduction occurs with multiple natural isolates. We further propose that this unusual mode of unisexual reproduction occurs in nature in parallel with normal bisexual reproduction.

Uniparental progeny completely lack signs of recombination between the two parents

As mentioned previously, H99α (as well as the H99α-derived strain VYD135α) and Bt63**a** have approximately 0.5% genetic divergence. The occurrence of uniparental reproduction in the VYD135α x Bt63**a** cross allowed us to test if the two parental genomes recombine with each other during development. We subjected some of the VYD135α x Bt63**a**, as well as the H99α x Bt63**a**, progeny to whole-genome sequencing. As expected, for the H99α x Bt63**a** cross, both parents contributed to the nuclear composition of their progeny, and there was clear evidence of meiotic recombination as determined by variant analysis (Figure 3B). When the VYD135α x Bt63**a** progeny were similarly analyzed, the nuclear genome in each progeny was found to be inherited exclusively from only the VYD135α parent (Figure 3C and S5), and the progeny exhibited sequence differences across the entire Bt63**a** genome. In contrast, the mitochondrial genome was inherited exclusively from the Bt63**a** parent, in accord with the PCR assay results discussed above. Additionally, the whole-genome sequencing data also revealed that while most of the H99α x Bt63**a** F1 progeny exhibited aneuploidy, the genome-shuffle strain VYD135α x Bt63**a** progeny were euploid (Figure S6A-B), and based on flow cytometry analysis these uniparental F1 progeny were haploid (Figure S6C).

The F1 progeny from crosses involving IUM96**a** as the *MAT***a** partner were also sequenced. Similar to the Bt63**a** analysis, the H99α x IUM96**a** F1 progeny exhibited signs of meiotic recombination, whereas the VYD135α x IUM96**a** F1 progeny did not (Figure S7). Congruent with the mating-type analysis, the progeny exclusively inherited nuclear genetic material from only one of the two parents. Furthermore, the H99α x IUM96**a** progeny were

found to be aneuploid for some chromosomes while the progeny of VYD135α x IUM96**a** were completely euploid (Figure S8). We also sequenced four progeny from basidium 7 from the H99α x IUM96**a** cross, which were suspected to be uniparental progeny based on mating-type PCRs. This analysis showed that all four progeny harbored only H99α nuclear DNA and had no contribution from the IUM96**a** genome further confirming the conclusion that uniparental reproduction occurs in wild-type crosses (Figure S7A). Combined, these results suggest the occurrence of a novel mode of sexual reproduction in *C. neoformans,* where two parents participate but the two parental genomes do not recombine with each other.

Meiotic recombinase Dmc1 is important for uniparental sporulation

Because the genomes of the uniparental progeny did not show evidence of meiotic recombination between the two parents, we sought to test whether the uniparental sporulation involves meiosis. We, therefore, tested whether Dmc1, a key component of the meiotic machinery, is required for the uniparental sporulation. The meiotic recombinase gene *DMC1* was deleted in congenic H99α, VYD135α, and KN99**a,** and mutant strains were subjected to mating. A previous report documented that *dmc1*Δ bilateral crosses display significantly reduced, but not completely abolished, sporulation in *Cryptococcus* (Lin et al. 2005). We observed a similar phenotype with the H99α *dmc1*Δ x KN99**a** *dmc1*Δ cross. While most of the basidia were devoid of spore chains, a small percent (21/760=2.7%) of the population bypassed the requirement for Dmc1 and produced spores (Figure 4A and S9A). When dissected, the germination rate for these spores was found to be very low with spores from many basidia not germinating at all (Table 2). Furthermore, *MAT*-specific PCRs revealed that some of the progeny were aneuploid. For VYD135α *dmc1*Δ x KN99**a** *dmc1*Δ, much fewer basidia (~0.1%) produced spore chains as compared to ~1% sporulation in VYD135α x KN99**a** (Figure 4A, B and S9B). Dmc1 mutant unilateral crosses sporulated at a frequency of 0.4% suggesting that only one of the parental strains was producing spores (Figure 4B). When a few sporulating basidia from multiple mating spots were dissected, two different populations of basidia emerged, one with no spore germination, and the other with a high spore germination rate and uniparental DNA inheritance (Table 2). Combined together, the *DMC1* deletion led to a 20-fold reduction in viable sporulation, observed as a 10-fold decrease from sporulation events in the bilateral mutant cross and a further 2-fold reduction in the number of basidia producing viable spores.

To further support these conclusions, *DMC1* was deleted in mCherry-H4 tagged KN99**a** and crossed with GFP-H4 tagged VYD135α. We hypothesized that GFP-H4 tagged VYD135α would produce spore chains in this cross because it harbors *DMC1* whereas mCherry-H4 tagged KN99**a**, lacking *DMC1*, would fail to do so. Indeed, all 11 observed basidia with only the GFP-H4 fluorescence signal were found to produce spores but only 2 out of 19 mCherry-H4 containing basidia exhibited sporulation (Figure S10). These results combined with spore dissection data show that Dmc1 is critical for uniparental sporulation.

Discussion

Hybridogenesis and parthenogenesis are mechanisms that allow some organisms to overcome some hurdles of sexual reproduction and produce hemiclonal or clonal progeny (Hörandl 2009, Avise 2015, Lavanchy and Schwander 2019). However, harmful mutations are not filtered in these processes, making them disadvantageous during evolution and thus restricting the occurrence of these processes to a limited number of animal species (Lavanchy and Schwander 2019). In this study, we discovered and characterized the occurrence of a phenomenon in fungi that resembles hybridogenesis. Fungi are known to exhibit bisexual, unisexual, and asexual reproduction and can switch between these reproductive modes depending on environmental conditions (Heitman et al. 2013, Heitman 2015). The discovery of hybridogenesis further diversifies known reproductive modes in fungi, suggesting the presence of sexual parasitism in this kingdom.

Hybridogenesis requires the exclusion of one of the parents, which is followed by endoreplication of the other parent's genome and meiosis to produce hemiclonal progeny. A similar process was found to occur in a human fungal pathogen, *C. neoformans*, in this study. The whole-genome sequence of the progeny revealed the complete absence of one parent's genome, suggesting manifestations of genome exclusion during hyphal growth. The mechanism of genome exclusion during hybridogenesis in animals is not entirely understood, except for a few insights from triploid fishes of the genus *Poeciliopsis* and water frogs, *Pelophylax esculentus*. Studies using *Poeciliopsis* fishes showed that haploid paternal genome exclusion takes place during the onset of meiosis via the formation of a unipolar spindle, and thus only the diploid set of maternal chromosomes is retained (Cimino 1972a, Cimino 1972b). On the other hand, studies involving *P. esculentus* revealed that genome exclusion occurs during mitotic division, before meiosis, which is followed by endoreplication of the other parental genome (Tunner and Heppich 1981, Heppich et al. 1982,

Tunner and Heppich-Tunner 1991). A recent study, however, proposed that genome exclusion in *P. esculentus* could also take place during early meiotic phases (Dolezalkova et al. 2016). Using fluorescence microscopy, we examined the steps of nuclear exclusion in *C. neoformans* and found that it occurs during mitotic hyphal growth and not during meiosis. We also observed that genome exclusion could happen with either of the two parents in *C. neoformans*, similar to what has also been reported for water frogs (Uzzell et al. 1976, Vinogradov et al. 1991, Holsbeek and Jooris 2009). Taken together, these results indicate that the mechanism might be at least partially conserved between these two distantly related organisms. The amenability of *C. neoformans* to genetic manipulation will aid in deciphering some of the unanswered questions related to hybridogenesis in animals.

The occurrence of hybridogenesis might also have significant implications for *C. neoformans* biology. Most (>95%) of *Cryptococcus* natural isolates belong to only one mating type, α (Zhao et al. 2019). While the reason behind this distribution is unknown, one explanation could be the presence of unisexual reproduction in the sister species *C. deneoformans* and *C. gattii* (Fraser et al. 2005, Lin et al. 2005, Phadke et al. 2014). The presence of a hybridogenetic mechanism in *C. neoformans* might help explain the mating-type distribution pattern for this species specifically. In this report, one of the *MAT***a** natural isolates, Bt63**a**, did not contribute to uniparental sporulation and the other isolate, IUM96**a**, produced uniparental spores in only one basidium, while the rest of the basidia produced *MAT*α progeny. We hypothesize that *MAT***a** isolates may be defective in this process due to either a variation in their genomes or some other as yet undefined sporulation factor. As a result, hybridogenesis would result in the generation of predominantly α progeny in nature reducing the *MAT***a** population and thus favoring the expansion of the α mating-type population. Furthermore, we propose that hybridogenesis can occur between any two opposite mating-type strains as long as each one of them is capable of undergoing cell-cell fusion and at least one of them can sporulate. This mode of reproduction might act as an escape path from genomic incompatibilities between two related, yet karyotypically-incompatible isolates and allow them to produce spore progeny for dispersal and infection.

The fungal kingdom is one of the more diverse kingdoms with approximately 3 million species. The finding of hybridogenesis hints towards unexplored biology in this kingdom that might provide crucial clues for understanding the evolution of sex. Fungi have also been the basis of studies focused on understanding the evolution of meiosis, and the presence of genome reduction, as well as the para-sexual cycle in fungi, have led to the

proposal that meiosis evolved from mitosis (Hurst and Nurse 1991, Wilkins and Holliday 2009). Hybridogenesis may be a part of an evolutionary process wherein genome exclusion followed by endoreplication and meiosis was an ancestral form of reproduction that preceded evolution of bisexual reproduction. Evidence supporting such a hypothesis can be observed in organisms undergoing facultative sex or facultative parthenogenesis (Booth et al. 2012, Fields et al. 2015, Hojsgaard and Harandl 2015, Hodač et al. 2019). The presence of these organisms also suggests that a combination of both sexual and clonal modes of reproduction might prove to be evolutionarily advantageous.

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Methods

C. neoformans wild-type strains H99α and KN99**a** served as the wild-type isogenic parental lineages for the experiments, in addition to *MAT***a** strains Bt63**a** and IUM96-2828**a**. Basidia-specific spore dissections were performed after two-five weeks of mating, and the spore germination rate was scored after five days of dissection. Strains and primers used in this study are listed in Table S3 and S4, respectively. See Supplementary methods for details. The sequence data generated in this study were submitted to NCBI with the BioProject accession number PRJNA682203.

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Table 1. Genotype analysis of basidia-specific spores germinated from H99α x KN99a and VYD135α x KN99a crosses.

Basidia #	H99α x KN99**a** cross				VYD135α x KN99**a** cross			
	Spores germinated/ dissected	**% germinated**	***MAT***	**Mito**	**Spores germinated/ dissected**	**% germinated**	***MAT***	**Mito**
1	5/14	36	4α + 1**a**	**a**	12/24	50	All α	**a**
2	14/14	100	7α + 7**a**	**a**	6/10	60	All α	**a**
3	12/14	86	2α + 7**a** +3**a**/α	**a**	15/15	100	All **a**	**a**
4	10/14	71	4α +6**a**	**a**	22/27	81	All **a**	**a**
5	7/13	54	6**a** + 1**a**/α	**a**	3/12	25	All α	**a**
6	13/14	93	6α + 7**a**	**a**	25/27	93	All α	**a**
7	11/14	79	6α + 5**a**	**a**	4/4	100	All α	**a**
8	14/14	100	12α + 2**a**	**a**	10/13	77	All α	**a**
9	10/14	71	4α + 6**a**	**a**	13/15	87	All α	**a**
10	14/14	100	7α + 7**a**	**a**	31/61	51	All α	**a**
11	14/14	100	10α + 4**a**	**a**	10/10	100	All **a**	**a**
12	12/14	86	8α + 4**a**	**a**	4/5	80	All **a**	**a**
13	4/11	36	All **a**	**a**	24/28	86	All **a**	**a**
14	13/13	100	8α + 5**a**	**a**	16/28	57	All **a**	**a**
15	14/14	100	7α + 7**a**	**a**	11/11	100	All **a**	**a**
16	14/14	100	6α + 8**a**	**a**	10/22	45	All α	**a**

Table 2. Genotype analysis of basidia-specific progeny from H99α *dmc1*Δ x KN99a *dmc1*Δ and VYD135α *dmc1*Δ x KN99a *dmc1*Δ crosses.

Basidia #	H99α *dmc1*Δ x KN99**a** *dmc1*Δ cross				VYD135α *dmc1*Δ x KN99a *dmc1*Δ cross			
	Spores germinated/ dissected	**% germinated**	***MAT***	**Mito**	**Spores germinated/ dissected**	**% germinated**	***MAT***	**Mito**
1	7/24	29	2α + 5**a**/α	**a**	0/26	0	-	-
2	2/20	10	All α	**a**	7/14	50	All **a**	**a**
3	3/20	15	All α	**a**	0/10	0	-	-
4	5/14	36	All **a**/α	**a**	8/18	44	All **a**	**a**
5	3/11	27	All **a**	**a**	12/12	100	All **a**	**a**
6	0/12	0	-	-	7/8	88	All **a**	**a**
7	7/26	27	All **a**	**a**	14/14	100	All **a**	**a**
8	0/9	0	-	-	0/8	0	-	-
9	0/19	0	-	-	0/14	0	-	-
10	1/11	9	α	**a**	19/19	100	All **a**	**a**
11	24/27	89	12α + 6**a** + 6**a**/α	**a**	0/5	0	-	-
12	5/22	23	1α + 4**a**/α	**a**	-	-	-	-

Figure Legends

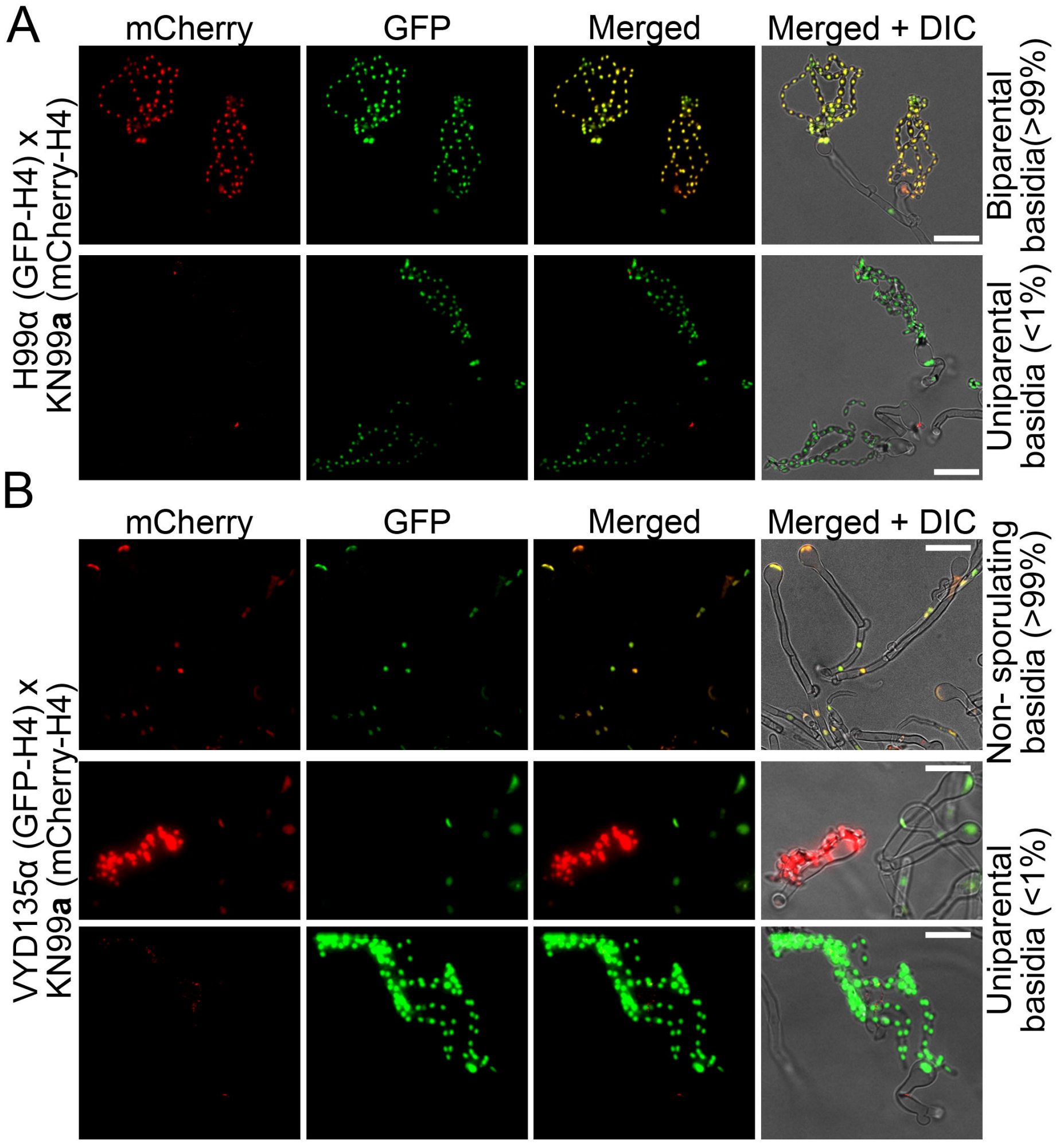
Figure 1. Fluorescence microscopy reveals uniparental sporulation in the wild-type crosses. (A) Mating of GFP-H4 tagged H99α and mCherry-H4 tagged KN99**a** revealed the presence of both fluorescent markers in most spore chains along with uniparental sporulation events in rare cases (<1%). In these rare sporulating basidia, only one of the fluorescent markers was observed in the spore chains, reflecting the presence of only one parental nucleus in these basidia. **(B)** Crosses involving GFP-H4 tagged VYD135α, and mCherry-H4 tagged KN99**a** revealed the presence of spore chains with only one fluorescent color. In the majority of basidia that have both parental nuclei, marked by both GFP and mCherry signals, spore chains are not produced suggesting a failure of meiosis in these basidia. Bars, 10 µm.

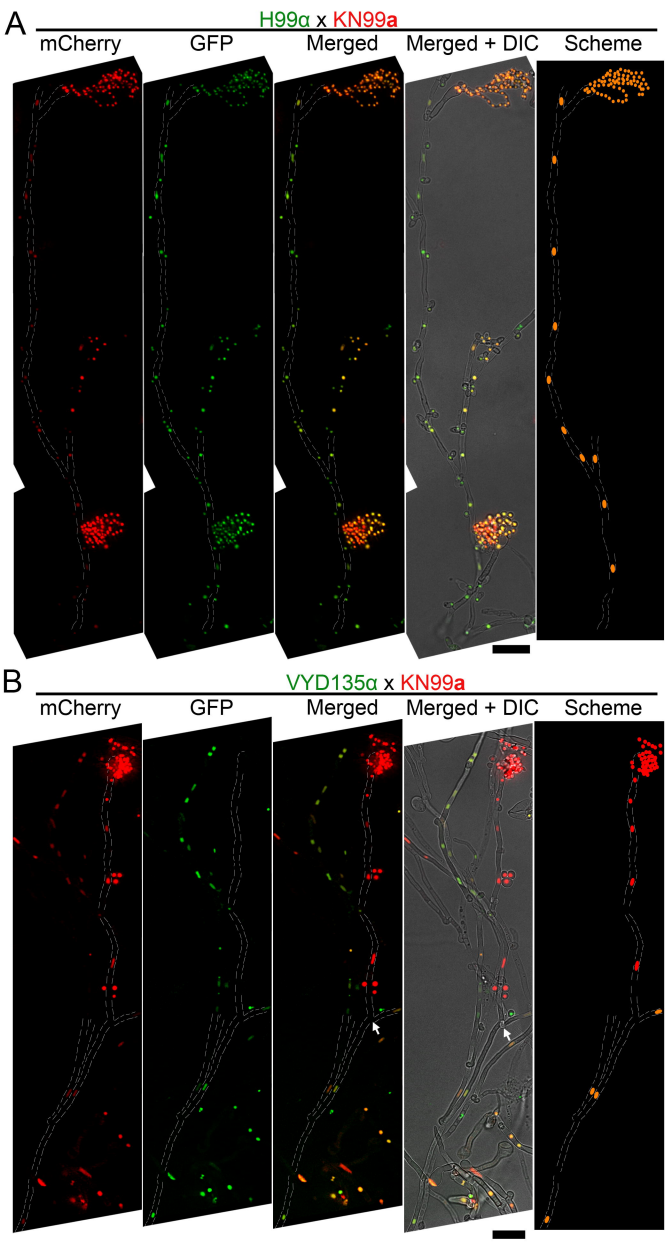
Figure 2. Pan-hyphal microscopy reveals the loss of one parental nucleus during unisexual reproduction. Spore-producing long hyphae were visualized in both (**A**) wild-type H99α x KN99**a** and (**B**) VYD135α x KN99**a** crosses to study the dynamics of nuclei in hyphae. Both nuclei were present across the hyphal length in the wild-type and resulted in the production of biparental spores. On the other hand, one of the nuclei was lost during hyphal branching in the VYD135α x KN99**a** cross and resulted in uniparental spore production. The arrow in **B** marks the hyphal branching point after which only one of the parental nuclei is present. The images were captured as independent sections and assembled to obtain the final presented image. Bars, 10 µm.

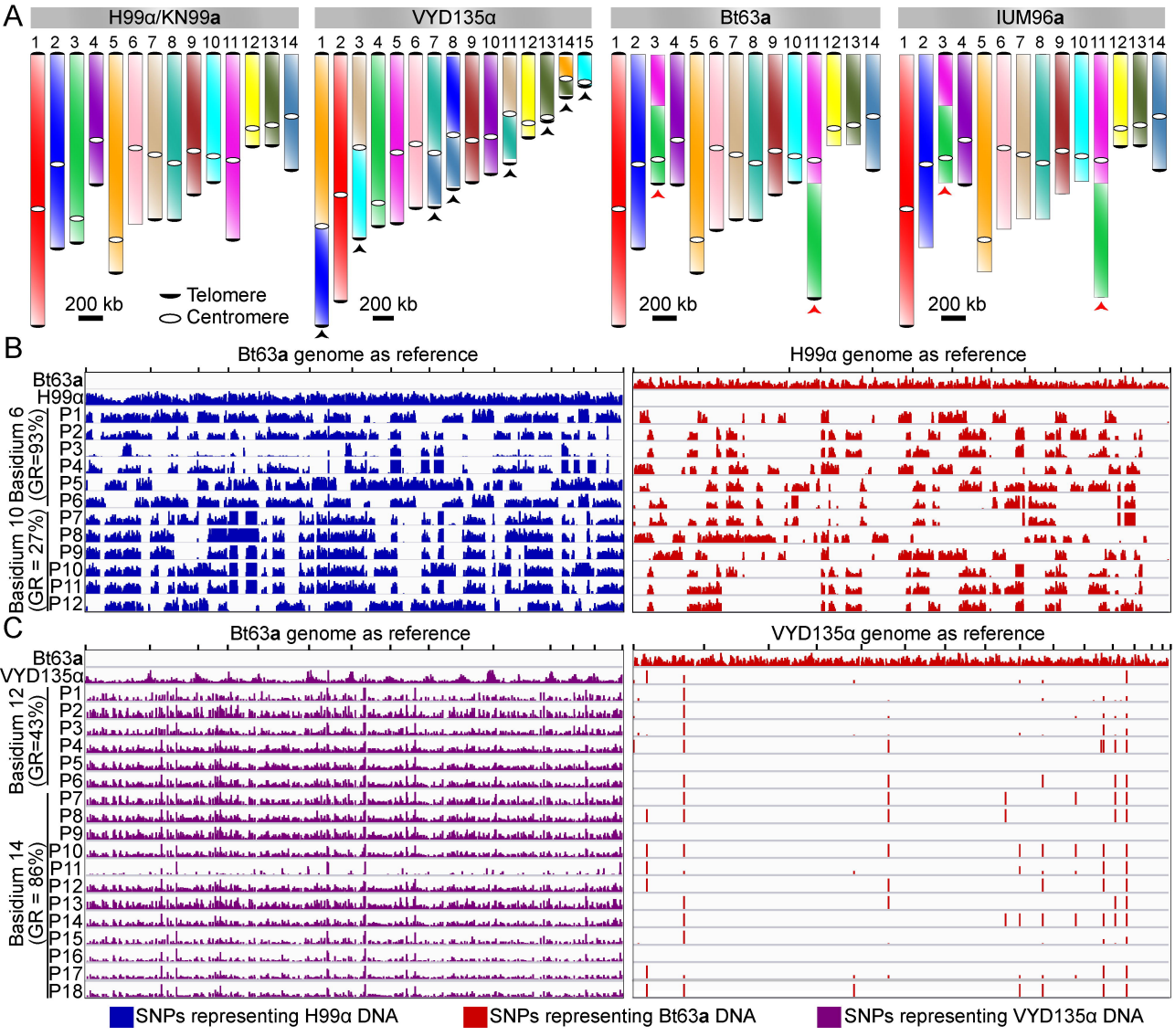
Figure 3. VYD135α progeny exhibit strict uniparental nuclear inheritance and lack the signature of meiotic recombination. (A) Chromosome maps for H99α/KN99**a**, VYD135α, Bt63**a**, and IUM96-2828**a** showing the karyotype variation. The genome of the wild-type strain H99α served as the reference. Black arrowheads represent chromosome translocations between VYD135α and H99α whereas red arrowheads mark chromosomes with a translocation between H99α and Bt63**a** or IUM96-2828**a**. **(B)** Whole-genome sequencing, followed by SNP identification, of H99α x Bt63**a** progeny revealed evidence of meiotic recombination in all of the progeny. The left panel shows SNPs with respect to the Bt63**a** genome whereas the right panel depicts SNPs against the H99α genome. H99α and Bt63**a** Illumina sequencing data served as controls for SNP calling. **(C)** SNP analysis of VYD135α x Bt63**a** progeny revealed no contribution of the Bt63**a** parental genome in the progeny as evidenced by the presence of SNPs only against Bt63**a** (left panel) but not against the

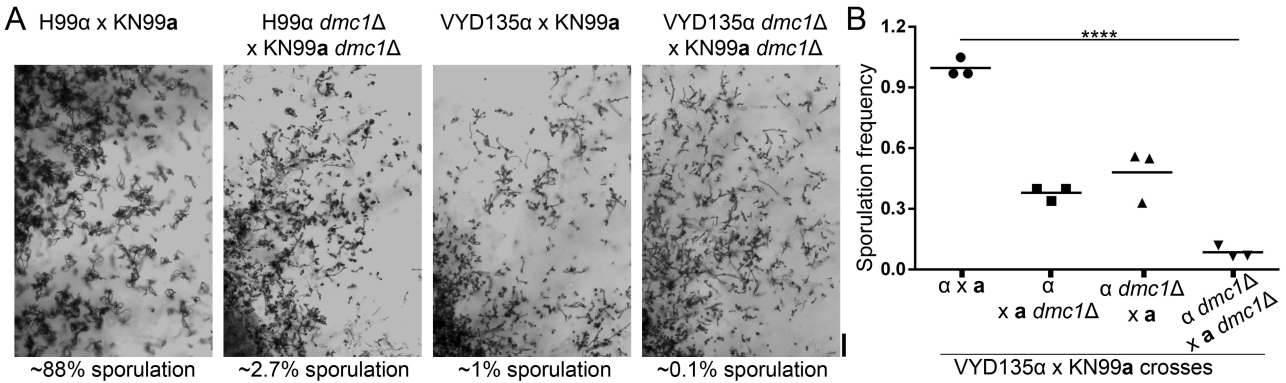
VYD135α genome (right panel). The presence of a few SNPs observed in VYD135α, as well as all VYD135α x Bt63**a** progeny, are within nucleotide repeat regions. GR stands for germination rate and P stands for progeny.

Figure 4. Meiotic recombinase Dmc1 is required for uniparental sporulation. (A) Light microscopy images showing the impact of *dmc1* mutation on bisexual and unisexual sporulation in *C. neoformans*. Bar, 100 µm. **(B)** A graph showing quantification (n=3) of sporulation events in multiple crosses with *dmc1*Δ mutants. At least 3000 basidia were counted in each experiment.


A
mCherry
GFP
Merged
Merged + DIC
H99α (GFP-H4) x
KN99**a** (mCherry-H4)
Biparental
basidia(>99%)
Uniparental
basidia (<1%)
B
mCherry
GFP
Merged
Merged + DIC
VYD135α (GFP-H4) x
KN99**a** (mCherry-H4)
Non- sporulating
basidia (>99%)
Uniparental
basidia (<1%)


A
H99α x KN99a
mCherry
GFP
Merged
Merged + DIC
Scheme
B
VYD135α x KN99a
mCherry
GFP
Merged
Merged + DIC
Scheme


A
H99α/KN99a
VYD135α
Bt63a
IUM96a
200 kb
Telomere
Centromere
B
Bt63a genome as reference
H99α genome as reference
Bt63a
H99α
Basidium 6 (GR=93%)
Basidium 10 (GR = 27%)
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
C
Bt63a genome as reference
VYD135α genome as reference
Bt63a
VYD135α
Basidium 12 (GR=43%)
Basidium 14 (GR = 86%)
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
P15
P16
P17
P18
SNPs representing H99α DNA
SNPs representing Bt63a DNA
SNPs representing VYD135α DNA


A
H99α x KN99**a**
H99α *dmc1*Δ x KN99**a** *dmc1*Δ
VYD135α x KN99**a**
VYD135α *dmc1*Δ x KN99**a** *dmc1*Δ
~88% sporulation
~2.7% sporulation
~1% sporulation
~0.1% sporulation
B
****
Sporulation frequency
1.2
0.9
0.6
0.3
0.0
α x **a**
α x **a** *dmc1*Δ
α *dmc1*Δ x **a**
α *dmc1*Δ x **a** *dmc1*Δ
VYD135α x KN99**a** crosses