Homothallic mating

A comparison of several mechanisms in yeasts

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Abstract

Although yeasts mainly reproduce asexually, many species are also able to have sex under specific conditions. In order to reproduce sexually a yeast cell usually mates with a cell of opposite mating type (heterothallism), but some species were discovered to reproduce also with cells of the same sex (homothallism). The molecular mechanisms of heterothallic reproduction have been studied well in several yeasts which revealed a number of general principles, but the homothallic reproduction strategies are less examined. The switch from heterothallic to homothallic mating in model yeast *Saccharomyces cerevisiae* differs a lot from the switch in *Candida albicans*, which suggests a lack of general principles. In this essay, I compare the mechanisms of heterothallic and homothallic mating in several Ascomycota and try to find similarities that can help elucidating the sexual cycles in less studied yeasts in future. Based on this comparison I suggest a classification system for the homothallic mechanisms in yeasts.

Biologie – Annie M.G. Schmidt

"Oh juffrouw Beekman, was u maar ééncellig
Dan kon de liefde u niet zo veel schelen
Dan zoudt u zich gewoon in tweeën delen
Ik geef wel toe: het is niet zo gezellig
Maar heel erg praktisch. Zo'n ééncellig wezen
Hoeft nooit een ander wezen te aanbidden;
Het deelt zich op een dag pardoes doormidden.
U kunt dat immers in de boeken lezen.

Terwijl u met u veertienbiljoen cellen
Zo treurig in de lunchroom zit te wachten
O, juffrouw Beekman 't is al over achten
Hij komt niet meer. Hij had toch kunnen bellen?

Hij komt niet meer. En toch, hij zei zo stellig.
Oh juffrouw Beekman, was u maar ééncellig.

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Introduction

All organisms in the tree of life show reproduction, but only the Eukarya can use sexual reproduction involving fusion of haploid gametes to produce offspring (Racquel Kim Sherwood, Scaduto, Torres, & Bennett, 2014). One eukaryotic kingdom is that of the fungi in which about 1.5 million species have been described (Butler, 2010). This enormous group of organisms is very diverse and comprises unicellular and multicellular organisms with different lifestyles and genetic backgrounds. It is therefore not surprising that there is a lot of variation among the reproduction mechanisms of fungi as well.

In this essay, I will focus on the sexual reproduction mechanisms of the unicellular fungi (yeasts) in the phylum Ascomycota. Most yeasts are able to produce progeny via both an asexual and sexual mechanism. Asexual reproduction is the most frequent and happens through budding or fission processes. It allows the yeast cell to pass its genes to the next generation without the need for a partner cell. Sexual reproduction does involve a partner. During sex, two haploid cells and their nuclei fuse to make one diploid cell. In the following process, called meiosis, the homologous chromosomes are segregated and the cell divides two times to produce four haploid cells. One specific characteristic of the Ascomycota is that these four haploid cells, or ascospores, are enveloped within an ascus (Lee, Ni, Li, Shertz, & Heitman, 2010). During meiosis, crossing over of genes between homologous chromosomes results in increased heterogeneity of the next generation. This is advantageous in a variable environment and thus increases fitness of the species. Sexual reproduction in yeasts is however often rare as it is induced only under specific conditions. Although mammals and plants are obligate sexual species, there are few yeasts that have been determined as strictly sexual (Ni, Feretzaki, Sun, Wang, & Heitman, 2011). On the other hand there are still many yeasts that are thought to be strictly asexual, but their number started to decrease in the last couple of years as our increasing knowledge of yeast mating systems led and leads to the discovery of sexual mechanisms in more species. One example of such a discovery is the well studied pathogenic yeast *Candida albicans*, that was thought to be strictly asexual for decades, but was found to reproduce sexually just a couple of years ago (Hull, Raisner, & Johnson, 2000).

The sexual reproduction strategies of yeasts and other eukaryotes share some general principles like mate recognition, cell-cell fusion yielding a zygote, meiosis and ploidy changes (Ni et al., 2011). One distinguishes two types of reproduction strategies, both including these principles; heterothallic and homothallic strategies. During heterothallic reproduction two individuals of opposite sex, or mating type, mate to produce progeny. A homothallic reproduction strategy however enables mating between individuals of the same sex (Ene & Bennett, 2014). Whereas almost every eukaryote that is able to have sex does this via a heterothallic strategy, only some yeasts and other fungi can additionally use homothallic strategies for sexual reproduction. Why homothallic mechanisms have developed in these organisms is not yet clear to evolutionary biologists as this kind of reproduction is likely to lead to in-breeding of a cell population and thus does not promote adaptation or increase fitness.

How yeasts exactly switch between their heterothallic and homothallic reproduction is clear for only a few species, including the well studied baker's yeast *Saccharomyces cerevisiae*. Cells of this model yeast can activate a homothallic mechanism that allows them to have sex with one of their own daughter cells, which are of the same mating type. This mechanism includes a switch of mating type in the mother cell (Haber, 2012). Interestingly, *C. albicans* cells are unable to switch mating type and use a completely different mechanism to activate homothallic reproduction (Alby & Bennett, 2010).

The differences between *S. cerevisiae* and *C. albicans* indicate that the molecular mechanisms underlying the transition from asexual to sexual reproduction and the switch between homothallism and heterothallism vary a lot among yeasts. What is known about these mechanisms in these two and other yeasts, and is there really no general system underlying these processes? How do the heterothallic and homothallic reproduction strategies compare between different species?

To answer these questions, I here explain and thoroughly compare the sexual reproduction and molecular heterothallic-homothallic switch mechanisms in *S. cerevisiae*, *C. albican*s and other unicellular Ascomycota. Based on this comparison, I suggest to divide the homothallic mating strategies of the unicellular Ascomycota into four groups. I distinguish two mating type switching mechanisms that are based on gene conversion and one that is based on inversion of a specific region of DNA. The fourth mechanism for homothallic mating is not based on mating type switching but on the activation of autocrine pheromone signalling. Furthermore, I will shortly discuss the possible reasons for the presence of homothallic mating mechanisms in yeasts, which is a hot topic among evolutionary biologists nowadays.

A better understanding of the molecular mechanisms, benefits and costs of the heterothallic and homothallic sexual reproduction systems could help us to elucidate similar mechanisms in less studied yeasts. Moreover, the knowledge might be useful in industry, or could help us to develop better applications to fungi that threaten health and agriculture.

The sexual cycle of *S. cerevisiae*

Heterothallic reproduction

S. cerevisiae mostly exists in a diploid stage, but its life cycle can also involve haploid stages (Knop, 2011). Haploid cells can be of two mating types, either mating type **a** or α. The mating type of a cell is genetically defined by its MAT locus, which contains either the idiomorph MATa or MATα, which correspond to mating type **a** and α respectively (Haber, 2012). Both diploid and haploid cells can reproduce asexually via budding, but the latter also have the ability to reproduce sexually. Nevertheless, the sexual cycles are rare and alternated with a large number of asexual reproduction cycles (Knop, 2011).

To attract a partner of opposite mating type for sex, **a** cells secrete **a** pheromone and α cells secrete α pheromone. The specific pheromones are sensed by G-protein coupled receptors (GPCRs) in the cell membrane of cells of the opposite mating type. There the pheromone induces several signal transduction pathways which have been extensively reviewed by Jones and Bennet (2011). The three main outputs of the pheromone signaling pathways are (a) the transcription of genes involved in mating, (b) the formation of projections (‘shmoos’) that grow specifically against the pheromone gradient and thus towards the prospective mating partner and (c) a G1 cell cycle arrest that allows the fusion of nuclei of two mating cells (Jones & Bennett, 2011).

The cellular and nuclear fusion of a haploid **a** and α cell result in a diploid **a**/α cell. The **a**/α cells reproduce asexually or undergo meiosis under starvation conditions to produce haploid **a** and α spores. The spores are resistant to harsh conditions, but can germinate when the conditions are good to form mature haploid yeast cells that are again capable of sexual reproduction (Butler, 2010). The germination completes the heterothallic cycle of *S. cerevisiae.*

To understand how **a**, α and **a**/α cells differ from each other I here explain the genetic make-up of the *S. cerevisiae* MAT locus (Figure 1A). In α cells, the MATα locus codes for transcription factors Matα1 and Matα2. Matα1 forms a complex with Mcm1, which is constitutively expressed, and together they activate α-specific genes that take care of production of α pheromone and Ste3, the GPC

receptor for **a** pheromone. Matα2 also interacts with Mcm1 to repress the **a**-specific genes that otherwise constitutively express **a** pheromone and Ste2, the receptor for α pheromone (Haber, 2012; Lee et al., 2010).

In **a** cells, the MATa locus expresses the transcription factor Mata1. The function of Mata1 in **a** cells is not known and cells lacking the entire MAT locus mate like **a** cells. Therefore, the **a** mating type is often also regarded as the 'default' mating type (Lee et al., 2010).

In **a**/α diploid cells the function of Mata1 becomes clear; Mata1 and Matα2, which are now both expressed, form an a1/α2 complex that activates diploid-specific genes and represses haploid-specific genes and the activity of Matα1, which leads to a decreased expression of α specific genes. As the **a** specific genes are in turn repressed by the Matα2-Mcm1 complex, the **a**/α cells are not able to mate (Lee et al., 2010). The a1/α2 complex also inhibits Rme1, a repressor of meiosis, to enable spore formation (Haber, 2012).

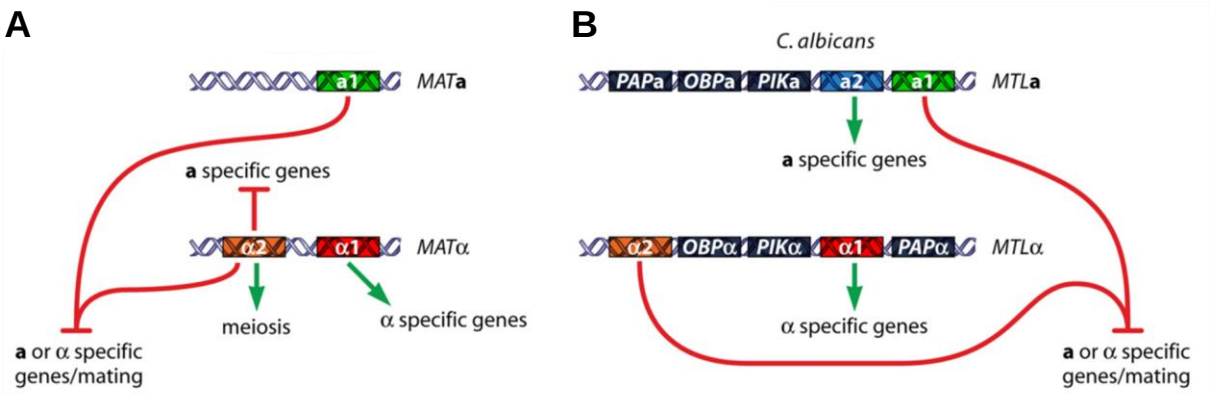

Fig. 1: **A)** Overview of genes in the *S. cerevisiae* MATa and MATα idiomorphs and **B)** *C. albicans* MTLa and MTLα idiomorphs. (From Lee et al., 2010)

Homothallic reproduction

Besides heterothallic reproduction, *S. cerevisiae* cells can also reproduce sexually by mating with cells of the same sex. To do so, the cell switches mating type (Figure 2). It therefore uses two silenced cassettes with copies of the mating type genes left and right of the MAT locus called HML and HMR respectively. The HML cassette contains copies of the α genes of the MATα idiomorph (HMLα) whereas the HMR cassette contains copies of the **a** genes that are found in the MATa idiomorph (HMRa) . The switch to the opposite mating type starts with cleavage of the active MAT locus, which contains either the MATa or MATα idiomorph, by HO endonuclease. This endonuclease introduces a double strand break (DSB) in the MAT locus. The HMRa and HMLα cassettes contain recognition sites for HO endonuclease as well but cannot be cleaved as these sites are sequestered by the nucleosomes involved in silencing of the cassettes (Haber, 2012). The DSB is bound by Rad51 proteins, that try to find homologous sequences to repair the DNA damage. The homologous sequences are found in either the HMLα or HMRa cassette and the MAT locus is repaired by copying genes from one of these loci, a process called gene conversion (Haber, 2012).

In **a** cells the DSB is repaired by gene conversion with the HMLα cassette in 90% of the time. Similarly, 90% of the time the MATα locus in α cells is repaired using the HMRa cassette (Weiler & Broach, 1992). Studies have shown that the cell's choice for a specific silenced cassette depends on the location rather than the content of the cassette as **a** cells with reversed silenced cassettes (HML**a** and HMRα) still use the HML locus for 90% of the repairs (Weiler & Broach, 1992). Between the HML locus and the MAT locus lies a recombination enhancer sequence (RE) which enhances recombination in the left arm of the chromosome and thus with the HML locus. However, in α cells

α2 and Mcm1 bind the RE and prevent this stimulation of recombination. As a consequence, **α** cells use the HMRa cassette more frequently for repair whereas **a** cells use the HMLα cassette (Coïc, Richard, & Haber, 2006).

After switching to the opposite mating type, a cell will have to find a partner of its original, now opposite, mating type to start a mating process as described for heterothallic reproduction. Homothallic mating is thus most efficient if only half of the haploid cells switch mating type. The ability of haploids cells to switch mating type depends on repression of the HO endonuclease by Ash1. During asexual budding Ash1 mRNA accumulates in the bud that will eventually form the daughter cell. Therefore, daughter cells are not able to switch mating type, while their mother cells, which represent half of the population, can (Long, 1997). HO endonuclease is also repressed in diploid **a**/**α** cells by the a1/α2 complex which prevents mating type switching in these cells (Haber, 2012).

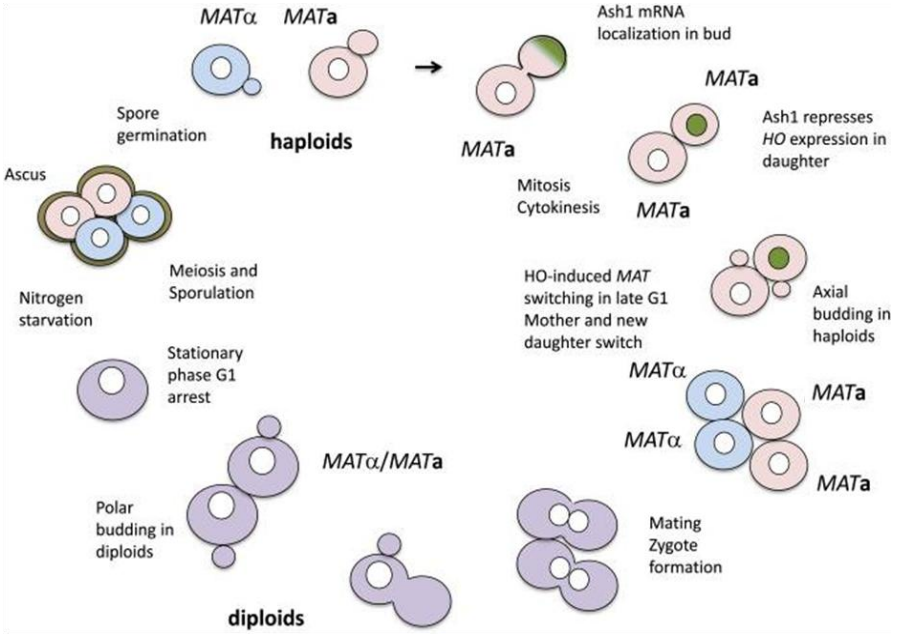

Fig. 2: Homothallic life cycle of *S. cerevisiae*. An **a** mother cell (pink) reproduces asexually. Ash1 mRNA asymmetric localization in the bud prevents mating type switching in the daughter cell (green) but not in the mother cell, which thus can switch to MATα (blue). The second daughter cell of the mother cell will be an **α** cell as well. The **α** cells and **a** cells can mate and form an **a**/**α** zygote that can subsequently sporulate to form four spores, which can germinate into four haploid cells under favorable conditions. (From Haber, 2012).

When to change reproduction strategy?

Although its molecular mechanisms of sexual reproduction and mating type switching have been studied quite well, it is still unclear which exact conditions make *S. cerevisiae* switch from asexual reproduction to sexual reproduction and from heterothallic to homothallic mating. It has been shown that certain environmental stresses can induce sexual reproduction and spore formation, which supports the idea that sex increases the chance of survival of a species (see 'Benefits of sex'). Limiting acetate conditions do not only induce sexual reproduction but can even make *S. cerevisiae* adapt parts of its sexual mechanism. Under such harsh conditions, an **a**/**α** cell appears to form two spores instead of four. The ascus of such a cell still contains four nuclei after meiosis, but two are degraded to save resources for maturation of the two remaining ones (Racquel K Sherwood & Bennett, 2009).

Whereas the transition between asexual and sexual reproduction seems to be influenced by the environmental conditions, the switch to homothallic reproduction specifically might also depend on the presence of prospective mating partners. Homothallic mating has less benefits than heterothallic mating and costs more energy because of the required mating type switching (See 'Benefits of sex'). It would thus make sense if cells do not switch mating type if there are cells of opposite mating type available for sex. In that case it is likely that the absence of 'opposite' pheromone is an additional requirement for effective HO endonuclease activity and thus mating type switching. Or in other words,

that the activity of HO endonuclease in a cell can be inhibited by pheromone of nearby prospective partners to ensure that the cell does not suddenly switch mating type. Something that contradicts this scenario is the fact that ‘opposite’ pheromone can *activate* homothallic mating in *C. albicans* (See 'The parasexual cycle of *C. albicans*: homothallic mating'),

The parasexual cycle of *C. albicans*

Another well-studied member of the Ascomycota is *C. albicans*, the most common fungal pathogen in humans causing mucosal and systemic infections (Alby, Schaefer, & Bennett, 2009). Sexual reproduction by this species has been discovered only a couple of years ago (Hull et al., 2000; Miller, Johnson, & Francisco, 2002). Subsequent research showed that the heterothallic reproduction mechanisms of this yeast are quite similar to that of *S. cerevisiae* but that its homothallic mating process is of a completely different kind.

Heterothallic reproduction

Unlike *S. cerevisiae*, mating-competent *C. albicans* cells are diploid and thus form a tetraploid cell after fusion. Meiosis has never been observed in *C. albicans* and the tetraploid cells return to a haploid, diploid or aneuploid stage via mitosis and subsequent loss of chromosomes. We therefore call the cycle ‘parasexual’ instead of sexual. The mechanism underlying the loss of chromosomes in the parasexual cycle is still unclear (Lee et al., 2010).

Chromosome 5 of *C. albicans* contains the mating type like locus (MTL), which contains either mating type idiomorph MTLa or MTLα. The MTL locus is related to the MAT locus in *S. cerevisiae*, but there are slight differences in the genes that the two idiomorphs encode and the function that some of them have (Figure 1B) (Hull et al., 2000). The MTLα idiomorph contains genes for expression of Mtlα1 and Mtlα2. Mtlα1 has the same function as Matα1, the up-regulation of **α**-specific genes, but Mtlα2 does not down-regulate **a**-specific genes, like Matα2 in *S. cerevisiae*. This is not surprising because repression of **a**-specific genes is not required as these are not constitutively expressed in *C. albicans*. Although the function of Mtlα2 in **α**/**α** diploid cells remains unclear, its function in **a**/**α** diploid cells is the same as in *S. cerevisiae* **a**/**α** cells: Mtlα2 forms an a1/α2 complex with Mtla1 to prevent the expression of **α**-specific genes thus making the **a**/**α** cells mating-incompetent (Lee et al., 2010; Tsong, Miller, Raisner, Johnson, & Francisco, 2003). Besides the transcription factor Mtla1, the MTLa idiomorph encodes an extra gene, *MTLa2*, which expresses the DNA-binding high-mobility group protein Mtla2. It is thought that Mtla2 is required for the activation of the **a**-specific genes in *C. albicans* (Tsong et al., 2003).

The MTL idiomorphs both contain three extra genes; a poly(A) polymerase gene (PAP), an oxysterol binding protein gene (OBP) and phosphatidylinositol kinase gene (PIK) (Butler et al., 2009). Whether these are involved in the mating process is not known.

It is clear that the genetic make-up of the mating type loci in *C. albicans* shows both differences and similarities with the *S. cerevisiae* system. Due to the a1/α2 complex diploid **a**/**α** cells are unable to mate and have to undergo homozygosis before they can enter the sexual cycle (Lee et al., 2010). After homozygosis the heterothallic mating process is similar to that in *S. cerevisiae* (Figure 3). The **a**/**a** and **α**/**α** cells secrete **a** and **α** pheromones, which are sensed by G-protein coupled receptors on the prospective mating partner and activate signal transduction pathways that induce formation of shmoos and fusion of cells. The zygote that is the product of heterothallic mating is an **a**/**a**/**α**/**α** tetraploid cell. These cells are not stable and therefore undergo mitosis and concerted chromosome loss which results in haploid (**a** or **α**), diploid (**a**/**a**, **a**/**α**, **α**/**α**) or aneuploid cells.

Homothallic reproduction

Besides the MTL locus there are no silenced copies of mating type genes like the HMLα and HMRa cassettes in *S. cerevisae* present in the *C. albicans* genome (Ni et al., 2011). *C. albicans* is therefore not able to switch mating type. To perform homothallic mating, this yeast uses an autocrine pheromone signaling system (Figure 3). Alby et al. showed that **a** cells can produce both **a** and α pheromone and that they produce Bar1, an aspartyl protease that degrades α pheromone and that is required for repression of the α pheromone secretion by **a** cells (Alby et al., 2009). Inactivation of Bar1 or high amounts of α pheromone trigger the production of α pheromone by **a** cells (Alby et al., 2009; Lee et al., 2010). The increase in α pheromone concentration is sensed by the Ste2 receptors on the **a** cell itself which activates a positive feedback loop that leads to a growing production of α pheromone by the cell. Eventually, the α pheromone will reach a concentration that can be sensed by nearby **a** cells as well, which will then form shmoos towards the cell. Subsequent fusion of the two **a** cells makes the homothallic mating a fact (Alby et al., 2009; Lee et al., 2010).

Bar1 thus has an important role in the activation of homothallic mating by *C. albicans*. The protease is also produced by *S. cerevisiae* **a** cells, but must have a different regulatory role in this species as *S. cerevisiae* **a** cells are not able to produce both **a** and α pheromones.

In contrast to **a** cells, *C. albicans* α cells do not express Bar1 and are only able to produce α pheromone. Still same-sex mating was also observed between cells of this mating type. The exact mechanism of homothallic mating by α cells remains unclear, although mating could be facilitated by the presence of mating-incompetent 'white' **a**/**a** cells, a phenomenon that will be discussed below (Alby et al., 2009; Tao et al., 2014).

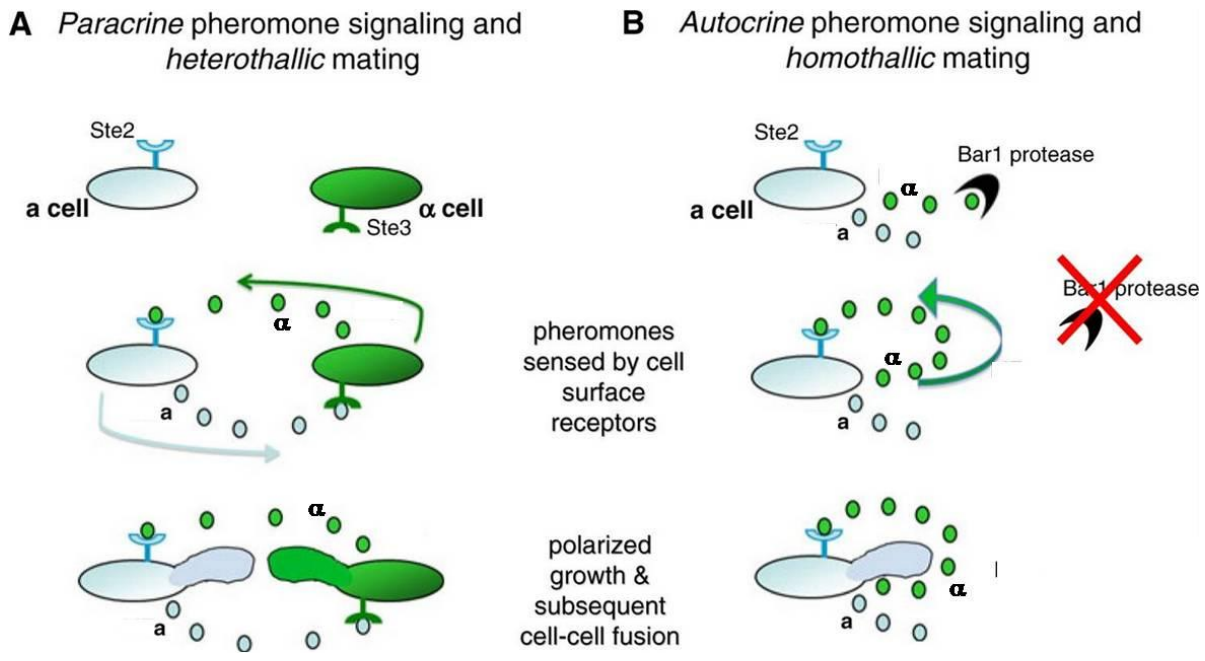

Fig. 3: Illustration of the heterothallic and homothallic mating mechanisms in *C. albicans*. A) Heterothallic mating is induced by paracrine pheromone signalling. This system is similar to heterothallic mating in *S. cerevisiae*. B) Inactivation of Bar1 in **a** cells leads to increased production of α pheromone via autocrine signalling, which results in auto-activation of the mating response and subsequent fusion with other **a** cells. (From: Alby & Bennet, 2010)

The white-opaque switch

Unlike other yeasts, *C. albicans* cells show a typical phenomenon called white-opaque switching that is linked to the mating process. Cells in the 'white' phase and cells in the 'opaque' phase differ in morphology and characteristics regarding virulence and growth (Alby & Bennett, 2010). Opaque cells are for instance more efficient than white cells in avoiding the immune system of the host (Alby & Bennett, 2010). Another important difference is that cells in the white phase are unable to mate.

Under certain conditions cells can switch between the white and opaque phase. The switch to the opaque phase is induced by external factors like temperature, oxygen and genotoxic and oxidative stress (Butler, 2010). It occurs epigenetically by a complex interaction network that involves six key regulators. One of the regulators, Wor1, is highly upregulated in opaque cells and is seen as the master regulator of the white-opaque switch (Hernday et al., 2013). In diploid **a**/**α** cells, the a1/α2 complex prevents the switch from white to opaque phase by repressing Wor1, the master regulator of the switch. Diploid **a**/**α** cells are thus always white and mating-incompetent, while diploid **a**/**a** and **α**/**α** cells can either be white and mating-incompetent or opaque and mating-competent.

Although homozygous white cells cannot mate, they seem to stimulate sexual reproduction. In the presence of pheromones from opaque cells of opposite mating type, the white cells form a robust biofilm (Alby & Bennett, 2010). The biofilms are thought to facilitate chemotropism of the opaque cells by protecting their pheromone gradients (Daniels, Srikantha, Lockhart, Pujol, & Soll, 2006). Furthermore, a recent study revealed that the homozygous white cells can also start to secrete pheromones themselves as a response on the pheromones of opaque cells, thereby promoting heterothallic *and* homothallic mating between surrounding opaque cells (Tao et al., 2014).

Up to now, the white-opaque switch has only been observed in *C. albicans* and *Candida dubliniensis* and does not seem to influence sexual reproduction in other yeasts. However, the existence of a mating-incompetent and mating-competent phase might be hard to notice if it is not accompanied by morphological differences. The fact that many species contain a homolog of *WOR1* also suggests that the two phase system is or was present in more yeasts. However, the function of Wor1 appears to vary significantly per species (Alby & Bennett, 2010).

When to change reproduction strategy?

In natural isolates, the majority of *C. albicans* cells are heterozygous at the MTL locus and thus white. For the transition from asexual to sexual reproduction, cells thus need to undergo homozygosis and subsequently switch to the opaque phase. Although we know that environmental factors play a role in these processes, the exact initiation mechanisms underlying homozygosis and white-opaque switching are not clear.

Conditions that induce the switch from heterothallic to homothallic reproduction in opaque cells remain rather unexplained as well. It has been suggested that several environmental niches of *C. albicans* down-regulate Bar1 and thereby induce homothallism, as artificial down-regulation of Bar1 activity induced this as well (Alby et al., 2009). Furthermore, it is obvious that the presence of homozygous white cells and their biofilm formation stimulate both heterothallic and homothallic reproduction of opaque cells.

Sexual reproduction in *S. cerevisiae* related yeasts

Candida glabrata is a pathogenic yeast that is closely related to *S. cerevisiae* (Figure 4). Heterothallic reproduction has never been observed in *C. glabrata* populations, although the cells contain all the genes necessary for mating. Homothallic mating has not been observed as well, although one stud y showed that *C. glabrata* is able to switch mating type at a very low rate (Brockert et al., 2003). The mating type switching mechanism is probably the same as that in *S. cerevisiae* as the

C. glabrata genome contains three loci similar to the MAT, HMRa and HMLα cassettes in *S. cerevisiae*. The only difference is that the HMRa-like cassette is located at a different chromosome and is not silenced (Brockert et al., 2003; Butler, 2010). Mtla1 is therefore expressed in both **a** and **α** cells. Interestingly, Mtla1 activity seems to be regulated by an alternative mechanism that involves splicing, as Mtla1 splicing only occurs in **a** cells (Butler, 2010). The observation of mating type switching suggests but does not proof that *C. glabrata* is mating-competent. It might be that this species lost its ability to activate sexual reproduction as many *C. glabrata* isolates do not produce pheromones or show any response to them (Ene & Bennett, 2014).

Another yeast that uses mating type switching to enable homothallic sex is *Kluyveromyces lactis*. The mechanism underlying this switch differs from that in *S. cerevisiae*, as *K. lactis* contains a non-functional copy of the HO endonuclease gene (Rajaei, Chiruvella, Lin, & Aström, 2014). The *K. lactis* genome contains the MAT locus and the two silenced HMRa and HMLα cassettes. Like *S. cerevisiae*,

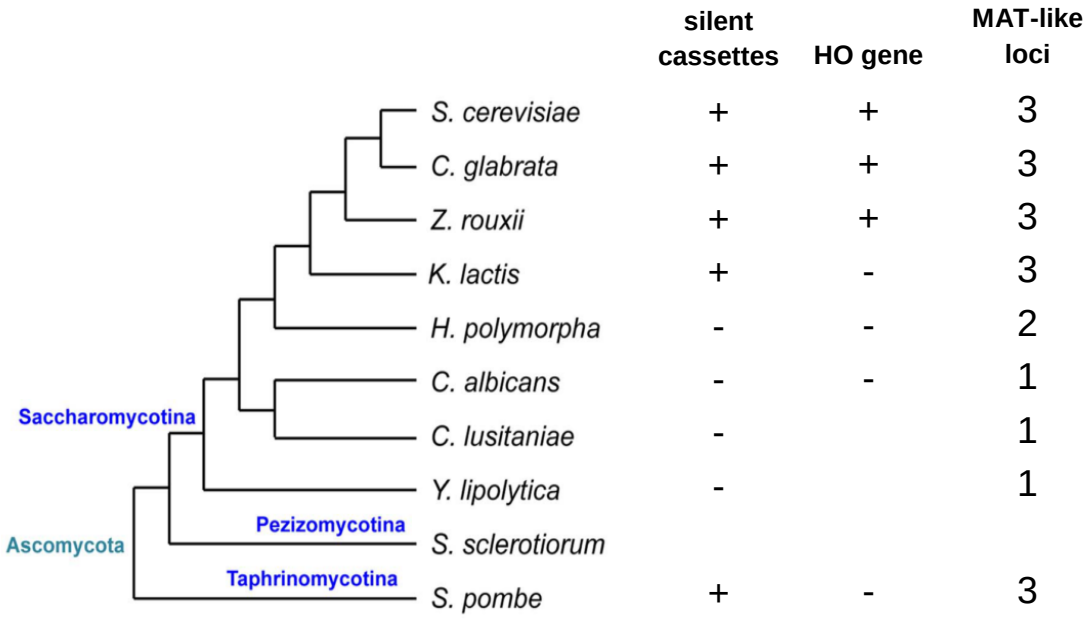

Fig. 4: Schematic of phylogenetic relationships between several Ascomycota yeast species. The presence of silent mating type cassettes and HO endonuclease and the number of mating type like loci in the species genome are also indicated. (Figure adapted from (Hanson et al., 2014; Maekawa & Kaneko, 2014)

it expresses proteins similar to Matα1, Matα2 and Mata1. However, a third gene is present in the MATα and HMLα cassette, namely *MATα3*, which encodes a transposase-like protein that plays a role in the switch from MATα to MATa. Under nutrient limiting conditions, the transcriptional regulator Mts1, a homolog of Rme1, is expressed. Mts1 binds close to *MATα3* and seems to recruit Matα3 proteins to induce excision of *MATα3*. The resulting gap is repaired by gene conversion which leads to the switch to MATa (Barsoum, Martinez, & Aström, 2010; Rajaei et al., 2014). The Mts1 regulator is also involved in the MATa to MATα switch, but here it activates the expression of hAT transposase 1 (Kat1) which cleaves the MATa locus at two sites. The resulting DSBs promote gene conversion with the HMLα cassette (Rajaei et al., 2014). In heterozygous **a**/**α** cells, the a1/α2 complex represses the expression of Mts1 (Barsoum et al., 2010).

Very recently, one elucidated a remarkable molecular mechanism for homothallic mating in the methylotrophic yeast *Hansenula polymorpha* (Hanson, Byrne, & Wolfe, 2014; Maekawa & Kaneko, 2014). The *H. polymorpha* genome contains two mating type loci, whereas most species have one or three mating type loci. The first locus contains homologous sequences of *MATa1* and *MATa2* and the

second locus those of *MATα1* and *MATα2* (Hanson et al., 2014). One locus is always transcriptionally repressed while the other remains active and determines the mating type of the cell. Which of the two loci is active depends on the orientation of the *MATa* and *MATα* genes and the 19 kb region that lies between them. To be able to mate with the same sex, cells can switch mating type via inversion of this section of the chromosome, which swaps the activity of the MAT loci (Figure 5). Nutrient limiting conditions strongly induce the frequency of switching (Hanson et al., 2014; Maekawa & Kaneko, 2014). This remarkable molecular switch has recently also been described in *Pichia pastoris* (Hanson et al., 2014).

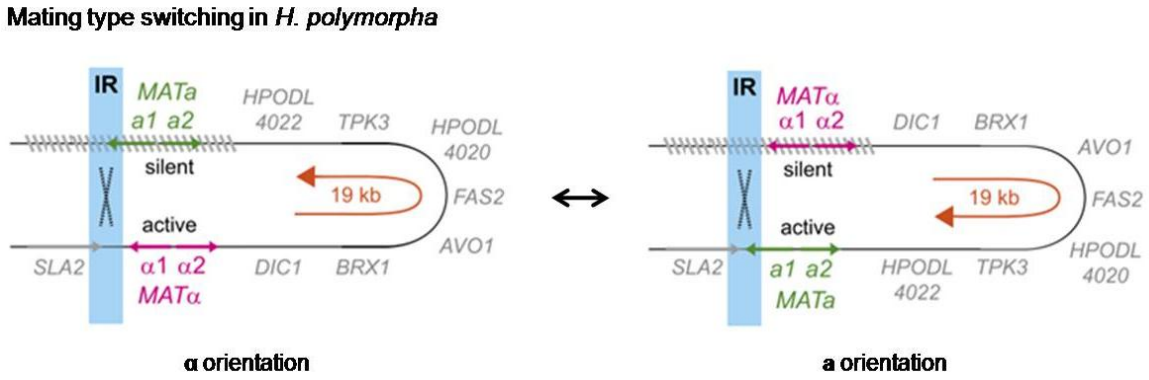

Fig. 5: Schematic of the mating type determination in *H. polymorpha*. *H. polymorpha* has two MAT loci (green and purple) of which one is active and one is silenced. Inversion of the two loci and their 19 kb region in between results in silencing of the originally active MAT locus and un-silencing of the opposite MAT locus. (Figure adapted from Hanson et al, 2014)

Sexual reproduction in *C. albicans* related yeasts

The yeast *Candida lusitaniae* is closely related to *C. albicans* and has a similar organization of mating type genes (Figure 4). However, it is thought that *C. lusitaniae* shows heterothallic reproduction via a sexual cycle including meiosis rather than a parasexual cycle, as spores are formed after mating (Reedy, Floyd, & Heitman, 2009). As *C. lusitaniae* lacks a number of important meiotic genes that are retained in *C. albicans*, *C. albicans* mating might involve a yet unrecognized form of meiosis (Reedy et al., 2009).

Another difference between the heterothallic mating mechanisms of *C. lusitaniae* and *C. albicans* is that the *C. lusitaniae* MTLα idiomorph does not contain *MTLα2*. An **a**/**α** diploid cell therefore lacks the a1/α2 complex that regulates transcription of mating type genes. Some studies suggest that Mtlα1 replaces Mtl*α2* by forming a repressor complex together with Mtla1 (Soll, Pujol, & Srikantha, 2009). If so, this function of Mtl*α*1 should also be considered in other yeasts.

Whether *C. lusitaniae* can mate via homothallic mechanisms remains unclear, but its genetic make-up suggests that such a system might look like the autocrine signaling mechanism in *C. albicans* (See 'A comparison of the sexual mechanisms in Ascomycota').

The diploid yeast *Lodderomyces elongisporus*, another *C. albicans* related species, seems to have lost the ability to reproduce via heterothallism as its genome lacks mating type genes (Butler, 2010). However, there are sequences for production of the α pheromone and Ste2 receptor, which probably enable this species to reproduce via an autocrine signaling system similar to homothallic mating by *C. albicans* (Alby et al., 2009; Lee et al., 2010). If this is true, *L. lactis* would be the first ascomycete that reproduces sexually without the presence of a MTL locus or any homologous stretch of genes (Alby & Bennett, 2010).

Sexual reproduction in a distantly related yeast

Last but not least I will shortly discuss the sexual reproduction mechanism of *Schizosaccharomyces pombe*. This fission yeast is distantly related to *S. cerevisiae* and *C. albicans* and can reproduce via heterothallic sex or homothallic sex after a mating type switch (Figure 4) (Hanson et al., 2014). At first sight the reproduction and switch system look a lot like those in *S. cerevisiae*, but in fact they differ in almost every detail.

S. pombe cells have mating type Plus or Minus and secrete pheromones that are homologous to **a** and α pheromone, respectively (Martin, Steenkamp, Wingfield, & Wingfield, 2012). The genome contains a mating type locus and two silenced donor sequences, but the three loci are unrelated to those in *S. cerevisiae* and other budding yeasts (Haber, 2012). *S. pombe* cells also produce a protein that degrades the α-like pheromone, like Bar1 does in *S. cerevisiae* and *C. albicans*. However, this protein, Sap30, is a serine carboxypeptidase and thus of a completely different enzyme family (Gonçalves-Sá & Murray, 2011).

S. pombe cells only respond to pheromones under nutritional stress conditions and their mating is always directly followed by sporulation (Hennig, Clemens, Rödel, & Ostermann, 2014; Merlini, Dudin, & Martin, 2013). *S. cerevisiae* cells however mate spontaneously forming diploid cells that reproduce asexually or sporulate upon nutritional stress and *C. albicans* cells do no sporulate at all (Merlini et al., 2013).

The mechanism of the mating type switch that *S. pombe* cells undergo to reproduce via homothallism differs a lot from that in other homothallic ascomycetes as well. There is no HO endonuclease and it is thought that an imprint creates a single nick in the mating type locus which is converted to a double strand break during replication (Haber, 2012; Ni et al., 2011). The created DSB is subsequently repaired by gene conversion using one of the silenced mating type loci.

S. pombe and the other Ascomycota parted ways in evolution a long time ago. The reproduction and switch mechanisms in *S. pombe* and *S. cerevisiae* have developed independently, but their similarities suggest they are a good example of convergent evolution (Haber, 2012).

The benefits of sex

It is not surprising that sexual reproduction cycles are alternated with a large number of asexual divisions as sex costs the cell a lot of energy (Goddard, Charles, Godfray, & Burt, 2005). What benefits of sex compensate these costs? The most recalled benefits are the possibility to produce recombinant offspring that is better adapted to the environment and the prevention of accumulation of harmful mutations (Ene & Bennett, 2014; Lee et al., 2010). For most yeasts, another benefit of mating is the formation of sexual spores, which are considered resistant to harsh conditions and thus increase the chance of survival (Billiard, López-Villavicencio, Hood, & Giraud, 2012). Sex can thus increase the survival of a species via several mechanisms.

C. albicans however lacks meiosis and cannot form ascospores. It has been suggested that this yeast prevents the formation of spores on purpose via its parasexual cycle as spores are often antigenic and thus increase the chance of detection of the yeast by the immune system of its host (Racquel K Sherwood & Bennett, 2009). *C. albicans* compensates for the disadvantage of a lower frequency of recombination that results from the lack of meiosis by utilizing aneuploid chromosomes for adaptation to environmental stresses (Lee et al., 2010). Whether meiosis in the Ascomycota is always followed by spore formation is unclear. If meiosis can also result in non-antigenic haploid cells, the suggested reason for development of a parasexual cycle in *C. albicans* voids.

The advantageous effect of recombination is obviously much less during homothallic mating than during heterothallic mating as partners are most times genetically identical. Still, some yeasts can only

reproduce sexually by homothallic mating or developed a homothallic mechanism besides their heterothallic mechanism. Why then did cells develop these homothallic mating mechanisms? As many species mainly reproduce asexually and as cells of opposite mating type are not always nearby, the lack of heterothallic sex could lead to a loss of fertility over time. Homothallism could thus be a way to protect the reproduction mechanisms in the cell until there are cells of opposite mating type available (Zeyl, 2009). Recombination is nevertheless probably still a second reason for same-sex mating. Smith et al. found that haploid cells that end up in the same ascus after meiosis rather mate with haploids from a different ascus than their own (Smith, Pomiankowski, & Greig, 2014). Mating between haploids of two different asci gives more heterogeneity after crossing over than mating between haploids in the same ascus. Thus, it seems that cells have developed mechanisms to maximize the effect of recombination during homothallism.

A comparison of the sexual mechanisms in Ascomycota

Unlike most eukaryotes, many yeasts are able to reproduce both asexually and sexually. As became clear through this essay many yeasts have loci like the *S. cerevisiae* MAT locus or *C. albicans* MTL locus to regulate their mating process. As a consequence their heterothallic reproduction systems have a lot of general principles and just a few species-specific differences. The exact genetic organization of the mating type loci has however changed over time in some species, which left the heterothallic mechanisms relatively unchanged but required development of quite distinct homothallic mating mechanisms which I suggest to divide into four groups.

The following five processes can be found in almost every yeast during the heterothallic mating process. A heterothallic cycle starts with the secretion of pheromone by a cell to attract potential mating partners. G-protein coupled receptors in cells of opposite mating type sense the pheromone and activate signal transduction pathways which results in activation and repression of mating genes. The cell then forms shmoos against the pheromone gradient and fuses with the pheromone secreting cell. The resulting diploid zygote can subsequently undergo meiosis to form haploid spores and complete the sexual cycle.

Only few organisms deviate from these main steps during mating, e.g. *C. albicans* that goes through a parasexual cycle lacking meiosis. However, as genetic recombination is observed during the parasexual cycle and as *C. lusitaniae* does not lack meiosis, there is still the possibility that *C. albicans* goes through a process similar to meiosis that still has to be discovered.

Although the headlines of each step in the mating process are the same, we observe differences in the details of some events. For instance, *C. albicans* **a** cells can produce both **a** and **α** pheromone while *S. cerevisiae* **a** cells are limited to pheromone **a** secretion. Nevertheless Bar1 ensures that the overall output of both species in the secretion process is the same by degrading the α pheromone around cells.

Small differences in the genetic make-up of the mating type loci most probably caused these variations in details between yeasts over time. Some species lost *MATa2* while others obtained new genes like *MATα3* (*K. lactis*) and the genes encoding PIK, PAP and OBP (*C. albicans*). Comparison of the mating type loci also shows that if genes are homologous their product does not necessarily has to fulfil the same task in the cell. Matα2 (Mtlα2) is for instance produced in both *C. albicans* and *S. cerevisiae* **α** cells but only functions as a repressor of **a** specific genes in the latter while its function remains unclear in the former. This has no effect on the overall output of the regulation as *C. albicans* cells do not express the **a** specific genes constitutively. Another example of a variation in the regulation mechanism can be found in *C. lusitaniae*. The a1/α2 complex prevents mating by **a**/**α** cells in most yeast species, but in *C. lusitaniae* Mtlα1 seems to have taken over the function of Mtlα2 in the

complex. Comparison of the heterothallic systems thus shows that small variations on the expression regulation mechanisms have developed but that the output of the system remained unchanged.

Unlike the heterothallic mating systems of yeasts, which seem relatively similar to each other, the homothallic mating systems reveal much bigger differences and do not fit into one standard mechanism. After comparison of the homothallic systems of these yeasts I conclude that the genetic organization of mating type loci plays a pivotal role in determination of the type of homothallic mechanism. I discerned four types of homothallic strategies that can be characterized by the evolutionary history of the species and its genetic organization of mating type loci (Table 1). Still several variations can be found within each type of mechanism that the yeasts might have developed as an extra adaptation to their specific niches.

Classification of homothallic strategies			
Type	**Nr. of mating type loci**	**Principle of mechanism**	**Ascomycota**
I	3	mating type switching by gene conversion	*S. cerevisiae* *C. glabrata* *K. lactis*
II	3	mating type switching by gene conversion	*S. pombe*
III	2	gene activity swap by DNA inversion	*H. polymorpha* *P. pastoris*
IV	1	autocrine pheromone signaling	*C. albicans* *L. elongisporus*

Table 1: Overview of the characteristics and members of the four types of homothallic strategies.

The first type of homothallic mechanism is found in yeasts that have three mating type loci similar to the MAT, HML and HMR loci in *S. cerevisiae*. After cleavage of the MAT locus by HO endonuclease, mother cells of these yeasts use the two silenced cassettes to switch mating type via gene conversion and to subsequently mate with their own daughter cells. The exact molecular events underlying the switch can differ less or more among species. In *C. glabrata* for instance, the activity of Mtla1 in the HMRa cassette seems to be regulated via splicing instead of silencing. Furthermore, *K. lactis* uses a transposase mechanism without HO endonuclease to induce the mating type switch. This also indicates that *K. lactis* uses alternative mechanisms to regulate the onset of mating type switching as HO endonuclease repression is impossible.

The homothallic strategy of *S. pombe* is classified as a second type of homothallic strategy even though cells have three mating type loci and switch mating type as well. The switching mechanism of *S. pombe* however differs too much in detail from that in *S. cerevisiae*, *C. glabrata* and *K. lactis*, which is not surprising as these yeasts are very distantly related. The α pheromone is for example degraded by Sap30 instead of Bar1, two proteins of different families. The homothallic systems have developed independently over time and the similarities between the systems are most likely just a result of convergent evolution (Haber, 2012). Thus, is seems that the evolutionary history of yeasts should be taken into account as well during classification.

A third type of mechanism to enable same-sex mating seems to be used by yeasts that have only two mating type loci and thus cannot switch by gene conversion. By smart use of chromosomal inversion these species can swap the activity or repression of the two mating type loci. This type of mechanism has already been described for *H. polymorpha* and *P. pastoris* and is presumably present in more methylotrophic yeasts or related species with only two mating type loci.

Yeasts with just one mating type locus in their genome cannot switch mating type as the genes of the opposite mating type idiomorph are not present in the cell's genome. They can therefore not use the three homothallic mechanisms described above, which suggests the existence of a fourth type of

mechanism. *C. albicans* is one of the few well-studied yeasts with a single MTL locus and studies revealed that this yeast uses autocrine pheromone signalling by opaque cells and paracrine signalling by nearby mating-incompetent white cells to activate homothallic mating. It is hard to say whether this mechanism is used by more yeasts with one mating type locus simply due to a lack of knowledge about these species. The fact that *L. elongisporus* also uses autocrine pheromone signalling for homothallic mating, suggests that this mechanism could indeed be more widely used by yeasts. Whether paracrine pheromone signalling by mating-incompetent cells occurs in other species is however unclear as the white-opaque system is rather unique to *C. albicans*.

The inactivation or absence of Bar1 seems to induce homothallic stragegies in *C. albicans*. This protease could perhaps be the key to elucidation of autocrine signalling mechanisms for homothallix mating in other yeasts with a single mating type locus, like *C. lusitaniae*. One should determine the presence and effect of this protease in these species.

Although yeasts mate by several types of mechanisms, they all do it for the same reason: increasing their chance of survival. Still many yeasts are thought to be asexual or their sexual mechanisms have not been described. The comparison and grouping of heterothallic and homothallic mating mechanisms of several yeasts that I presented here can help to determine the heterothallic and especially the homothallic mechanisms in these species. When the number of mating type loci in the genome of a species and its phylogenetic history have been determined one can already guess which of the four types of homothallic mechanisms that I distinguished here is used for same-sex mating. An analysis of the niche of the yeast could provide information about the details of the mechanism. The sexual strategies of species with the same type of homothallic mechanism and niche are likely to be similar to that of the species under examination and could also provide information. However, determining the exact sexual mechanisms in yeasts can be difficult as the comparison showed us that not any species uses exactly the same mechanism for mating. Differences in molecular details are abundant and many sexual strategies are not yet well understood. It would therefore not surprise me if more types of especially homothallic mechanisms will be found in future.

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