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SHORT REVIEW

Reproductive isolation in *Saccharomyces*

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Although speciation is one of the most interesting processes in evolution, the underlying causes of reproductive isolation are only partially understood in a few species. This review summarizes the results of many experiments on the reproductive isolation between yeast species of the *Saccharomyces sensu stricto* group. Hybrids between these species form quite readily in the laboratory, but, if given a choice of species to mate with, some are able to avoid hybridization. F1 hybrids are viable but sterile: the gametes they produce are inviable. For one pair of species, hybrid sterility is probably caused by chromosomal rearrangements, but for all the other species, the major cause of hybrid sterility is antirecombination—the inability of diverged chromosomes to form crossovers during F1 hybrid meiosis. Surprisingly, incompatibility between the genes expressed from different species' genomes is not a major cause of F1 hybrid sterility, although it may contribute to reproductive isolation at other stages of the yeast life cycle.
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Introduction

Humans have used *Saccharomyces cerevisiae* for thousands of years to make alcohol and to raise bread (Fay and Benavides, 2005). Yeast is also ideal for studying fundamental biology, and we can probably understand and control its cellular and molecular systems better than those of any other organism. In recent years, *S. cerevisiae* and closely related species have been used successfully for studies on experimental evolution, natural population genetics and speciation (Zeyl, 2006; Kuehne *et al*., 2007). In this review, I will focus on the reproductive barriers between the *Saccharomyces sensu stricto* species.

Yeast life history

The life cycle of *S. cerevisiae* has been defined in great depth by careful laboratory observations and experiments. When grown in rich medium, diploid cells reproduce asexually, frequently dividing by mitosis and budding off genetically identical cells. But when placed in medium lacking sufficient nitrogen to maintain mitosis diploids can undergo meiosis, producing a tetrad of four haploid spores. These spores are dormant and resistant, but when returned to rich medium, they germinate into metabolically active haploid gametes of two mating types, *MAT*α and *MAT***a**. Two gametes with different mating types can fuse together (this is called mating, but is analogous to fertilization in most higher organisms) to produce a single diploid cell, which can then divide by mitosis in the rich medium, completing the life cycle. Described like this, the yeast life cycle is not much different from that of higher organisms like fruit flies, in that there is a period of diploid clonal expansion forming a colony of yeast cells (analogous to the body of a fly), the production of two types of haploid gametes (analagous to sperms and eggs) and the fusion of haploid gametes to produce new diploids. But there are some significant differences in the life cycle and mating system of yeast compared with higher organisms.

Figure 1 shows the life cycle of yeast, starting with the fusion of two haploids to produce a diploid that then grows clonally by mitosis. Sex in yeast is facultative, not obligate, and not all diploids in population will enter meiosis when deprived of the nutrients required to sustain diploid mitosis. Some starving diploids that do not enter meiosis die, but others can survive in a low metabolic quiescent state for many months, utilizing intracellular nutrients, before resuming mitotic growth when the required nutrients are restored (Fabrizio and Longo, 2003). Meiosis and sporulation usually produces a tetrad of haploid spores, but it can also produce triads, dyads and monads of spores, depending on the conditions (Taxis *et al*., 2005). Diploids are heterozygous at the mating-type locus, *MAT*, so meiosis produces spores that are hemizygous for one or other mating-type allele. Spores are enclosed within an envelope (the ascus) and joined by interspore bridges (Coluccio and Neiman, 2004), so that when they germinate, they tend to mate with gametes produced by the same meiosis—a form of self-fertilization. Lab experiments find that most matings occur like this, and less than 1% of gametes fuse with gametes from other tetrads (Reuter *et al*., 2007). But the outbreeding rate increases when tetrads are eaten by animals whose guts digest the asci, but not the spores, releasing gametes from their tetrads, (Reuter *et al*., 2007). Other factors might also alter the outbreeding rate, for example, ascii containing only three viable spores will leave an unfertilized gamete that can fuse with one from another ascus. Also, an unfertilized gamete can divide by

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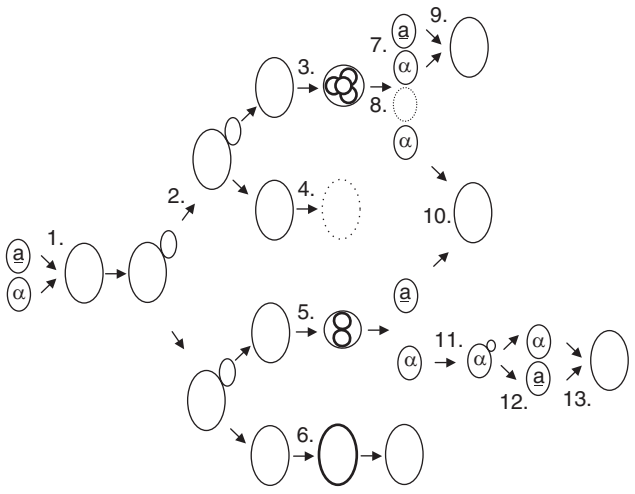

Figure 1 Yeast life cycle. (1) A diploid is formed by the fusion of a MAT**a** haploid and a MATα haploid. (2) Clonal expansion—diploids divide by mitosis until starved. (3) Starvation—most diploids undergo meiosis, forming a tetrad of haploid spores. (4) Starvation—some diploids die. (5) Starvation—some diploids undergo meiosis but produce fewer than four spores. (6) Starvation—some quiescent diploids remain viable at a low metabolic rate. (7) Germination—spores become metabolically active haploid cells. (8) Sterility—some spores are inviable. (9) Self-fertilization—some haploids fuse with other haploids from the same tetrad. (10) Outcrossing—some haploids fuse with haploids from other tetrads. (11) Haploid mitosis—haploids can divide by mitosis. (12) Mating-type switching—haploids that have divided can switch mating type. (13) Autodiploidization—haploids that have switched mating type can fuse with clone mates.

haploid mitosis, and having done so can change mating type, by physically changing the allele at the *MAT* locus to that encoding the other mating type. A cell that has switched mating type can then fuse with cell it previously produced, making a diploid that is completely homozygous except at *MAT*. If the gene responsible for this mating-type switching, *HO*, is inactivated by mutation, then clones of haploids can be propagated from single spores.

The relative roles and frequency of sporulation, selfing, haploid mitosis and mating-type switching are not well understood in natural populations, so care should be taken when assuming that the laboratory results are applicable to natural populations. That said, I will describe here yeast in an analogous way to most higher organisms, treating the diploid phase as dominant and haploidy as a transient phase containing gametes that are designed to be fertilized as soon as possible.

Saccharomyces sensu stricto species

There are currently six wild members of the *Saccharomyces sensu stricto* group, *S. cerevisiae*, *S. paradoxus*, *S. cariocanus*, *S. bayanus*, *S. kudriavzevii* and *S. mikatae* and a seventh domesticated species, *S. pastorianus* (also known as *S. carlsbergensis*) that is a hybrid species formed from *S. cerevisiae* and *S. bayanus* and found only in the strains used for the production of lager type beer (Naumov *et al*., 2000). Two species, *S. paradoxus* and *S. cariocanus*, have indistinguishable DNA sequences (Liti *et al*., 2006). These are also the most closely related species to *S. cerevisiae*, which shares with them an average nucleotide identity of 90% in coding genes and 80% in non-coding sequences (Kellis *et al*., 2003; Liti *et al*., 2006). The *Saccharomyces sensu stricto* species most distantly related to *S. cerevisiae* is *S. bayanus*, which is on average 80% identical in coding regions and 62% identical in non-coding regions (Kellis *et al*., 2003).

Two species, *S. cerevisiae* and *S. paradoxus*, are easily isolated from oak trees all around the world. As far as we can tell, they have very similar ecology—both can be isolated using the same protocol and they can be found in oak bark samples isolated within centimetres of each other (Sniegowski *et al*., 2002). The local population structure of *S. paradoxus* is dominated by clonality—the same genotype can be isolated again and again, with decreasing likelihood as the scale of sampling increases from a centimetre to a kilometre scale (Johnson *et al*., 2004; Koufopanou *et al*., 2006). On a continental scale, there appears to be free gene flow and recombination within *S. paradoxus* populations, but genetic exchange is cut off sharply between continents, and European, American and East Asian populations are genetically distinct, diverging by up to 4.6% at the sequence level (Koufopanou *et al*., 2006; Liti *et al*., 2006). This geographical structure is less strong in wild populations of *S. cerevisiae*, probably because of the confounding effect of association with humans (Liti *et al*., 2006; Aa *et al*., 2006). These population genetics studies as well as a recent phylogenetic analysis (Ruderfer *et al*., 2006) confirm the expectation that outcrossing in yeast is rare.

Premating reproductive isolation

An F1 hybrid diploid is formed when a haploid gamete from one species mates with a haploid gamete from another species. All six wild *sensu stricto* species can hybridize in the laboratory (Naumov *et al*., 2000). An interesting question is whether, given a choice, a gamete prefers to mate with its own species rather than with another species. Any such species recognition might reduce or prevent gene flow between species if they meet. *S. cerevisiae* and *S. paradoxus* are known to coexist in the same location in the wild, so mate preference could potentially contribute to premating reproductive isolation.

Murphy *et al*. (2006) investigated premating reproductive isolation in yeast by setting up mating trials with *S. cerevisiae* and *S. paradoxus*. The strains used were made heterothallic by replacing the *HO* gene with one of two antibiotic-resistance genes, allowing stable clones of pure *MAT***a** or *MAT*α gametes to be propagated. In each trial, a single vegetative haploid cell of known mating type and antibiotic resistance was placed in contact with two potential mates of the other mating type, one of the same species with the same antibiotic resistance and one of the other species with the other antibiotic resistance. The antibiotic resistance of the resulting diploids was used to determine whether they were hybrids or non-hybrids. No general tendency to avoid hybridization was detected. But other mate choice trials, which included the same strains as Murphy *et al*. (2006) along with several other pairs of strains, discovered a significant and consistent preference for mating with the same species (Maclean and Greig, 2008). The major difference between the experiments is that Maclean and Greig (2008) set up

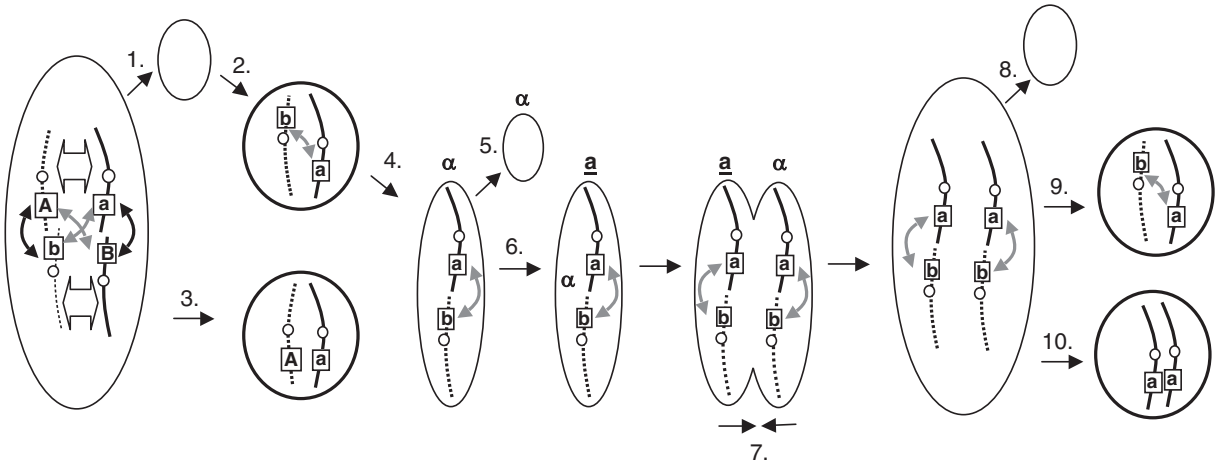

Figure 2 Potential postmating reproductive barriers. Figure shows how postmating reproductive isolation can potentially affect a hybrid at many different stages of the *Saccharomyces* life cycle. Two of the sixteen colinear chromosome pairs are shown. Dashed chromosomes from one species contain two interacting genes A and b, solid chromosomes from the other species contain two interacting genes a and B. Normal, compatible genetic interactions are shown with black arrows, hybrid, potentially incompatible interactions are shown with grey arrows. Chromosomal incompatibility (rearrangement or antirecombination) is represented by broad arrows. (1) Prevention of F1 hybrid mitosis. (2) Prevention of F1 meiosis or sporulation. (3) Failure of F1 meiosis to produce balanced gametes. (4) Prevention of F1 gamete germination. (5) Prevention of F1 gamete mitosis. (6) Prevention of F1 gamete mating-type switching. (7) Prevention of F1 gamete fusion (F2 zygote formation). (8) Prevention of F2 hybrid mitosis. (9) Prevention of F2 hybrid meiosis or sporulation. (10) Failure of F2 meiosis to produce balanced gametes. Chromosomal incompatibility (antirecombination or rearrangement) acts at stage 3, preventing all downstream stages (3–10). Speciation genes that are dominant (A and B) could potentially act at stages 1–3. Those that are recessive (a and b) could potentially act at stages 4–10.

the trials using wild-type spores instead of using haploids that had already germinated and been cultured as heterothallic vegetative cells. Thus, differences between the species in spore germination timing in the wild might produce an effective premating reproductive barrier. When given a choice of species as mates, discrimination was often near perfect, with hybridization only occurring when no choice of mate was available (Maclean and Greig, 2008). However, hybrids formed readily when no mate of the same species was offered. Furthermore, the possibility that mate preference was strain-specific rather than species-specific was not tested. It is possible that the genetic differences in germination and mating timing underlying mate preference also exist between different strains of the same species. Mate choice seems to be a relatively weak barrier between the *Saccharomyces* species.

Postmating reproductive isolation

The complexity of the yeast life cycle (Figure 1) means that there are several stages at which postmating reproductive isolation can potentially occur (Figure 2). To date, only some of these stages have been examined systematically. F1 hybrids appear normal: they divide by asexual budding and form spores by sexual meiosis. But F1 hybrids are sexually sterile—the spores they make are inviable. Fertility is measured by isolating individual spores from tetrads by micromanipulation and placing them onto a medium that usually promotes spore germination and colony formation. Greater than 90% of the spores produced by non-hybrids form colonies, but most hybrids produce only 1% or fewer viable spores. One known exception is the hybrid formed between *S. paradoxus* and *S. cariocanus*, which produces about 5% viable spores (Liti *et al*., 2006). The observed yeast hybrid sterility could potentially be caused by factors affecting the F1 hybrid meiosis, or hybrid spore germination or hybrid gamete mitosis (stages 3, 4, and 5 in Figure 2). The cause of this F1 hybrid sterility has been the subject of several research studies.

Chromosomal rearrangements

In many plant species, reproductive isolation is caused by translocations that rearrange the genome of one species relative to another. Chromosomal rearrangements between species prevent the gametes produced by an F1 hybrid meiosis from receiving a complete haploid set of genes (Coyne and Orr, 2004). Some of the *Saccharomyces sensu stricto* species differ by as many as four translocations, but others have none (Fischer *et al*., 2000; Kellis *et al*., 2003). *S. cerevisiae* and *S. paradoxus*, the species whose hybrids have been most studied, are almost perfectly collinear—there are four very small inversions, which could only reduce fertility in the rare event of a meiotic crossover occurring within them (Kellis *et al*., 2003). Chromosomal rearrangements cannot therefore explain hybrid sterility in these and other collinear *Saccharomyces* species.

Chromosomal rearrangements can, however, explain the sterility of hybrids between *S. paradoxus* and *S. cariocanus*. Assuming that unbalanced yeast gametes lacking part of a chromosome are inviable, but those gaining a duplicate part of a chromosome are viable, each reciprocal translocation should reduce hybrid spore viability to 50% of normal and each non-reciprocal translocation to 75% of normal. *S. paradoxus* and *S. cariocanus* differ by four large reciprocal translocations that should reduce fertility of their hybrids to 6% ($0.5^4 = 0.0625$) of normal, very close to that observed by Liti *et al*., 2006. The fact that the genome sequences of these species are indistinguishable (Liti *et al*., 2006) further supports the idea that chromosomal rearrange-

ment is the sole cause of the partial sterility of *S. paradoxus* and *S. cariocanus* hybrids.

But chromosomal rearrangement is insufficient to explain the sterility of any other yeast F1 hybrid. All the other yeast species have fewer (or no) rearrangements and produce hybrids that are more sterile than *S. paradoxus* and *S. cariocanus*. This was confirmed by Delneri *et al*. (2003), who engineered one or two reciprocal translocations into the genome of *S. cerevisiae* to make it collinear with one or other of two *S. mikatae* strains. Crosses between the engineered *S. cerevisiae* strains and wild-type *S. cerevisiae* strains showed the predicted reductions in fertility to 50 or 25% ($0.5^2 = 0.25$) of normal. But crosses between the *S. cerevisiae* strain with one engineered rearrangement and its collinear *S. mikatae* counterpart produced only a few hybrids with increased fertility, and these were found to be aneuploid. The other *S. cerevisiae* strain, with two engineered rearrangements, produced no fertile hybrids with its collinear *S. mikatae*. These data confirm that something other than chromosomal rearrangement is the major cause of yeast hybrid sterility.

Antirecombination

The mismatch repair system detects and corrects mismatched DNA base pairs. Mismatches are produced by mutation, during DNA replication, and when recombination is initiated between DNA molecules, which differ in sequence (Harfe and Jinks-Robertson, 2000). Homologous recombination requires the formation of an intermediate heteroduplex DNA structure containing complementary strands from the two recombining chromosomes. If these chromosomes are diverged, there will be mismatches in the heteroduplex DNA, which are detected by the mismatch repair system. Mismatches may be repaired, causing gene conversion, or recombination may be aborted, a phenomenon called antirecombination (Borts *et al*., 2000). In yeast, chromosomes must recombine with their homologues to assure proper meiotic segregation (Roeder, 1997). The formation of a physical connection between homologous chromosomes is thought to orient them on the meiotic spindle allowing proper chromosome disjunction. Mutations that reduce meiotic recombination also reduce fertility because non-disjunction results in aneuploid gametes, with some failing to receive a full haploid chromosome complement and others receiving extra chromosomes.

Could yeast hybrid sterility be caused by antirecombination between diverged chromosomes from different species? The rare surviving gametes produced by F1 hybrids between *S. cerevisiae* and *S. paradoxus* are highly aneuploid and their chromosomes are rarely recombinant (Hunter *et al*., 1996; Greig *et al*., 2002b), the expected effects of antirecombination. Interestingly, crosses between *S. cerevisiae* and *S. cariocanus*, which are probably reproductively isolated by chromosomal rearrangement (see above) serve as a natural experimental control. Their surviving gametes are euploid because their genomes are not sufficiently diverged for antirecombination to be effective (Liti *et al*., 2006). Simply by deleting genes involved in the mismatch repair system, Hunter *et al*. (1996) succeeded in increasing the hybrid fertility 10-fold. Crosses between diverged members of the same species also have reduced fertility that is caused, at least in part, by the action of the mismatch repair system (Greig *et al*., 2003) and there is a continuous relationship between divergence and reproductive isolation (Liti *et al*., 2006). The inability of diverged chromosomes to recombine provides the only explanation for yeast F1 hybrid sterility that has direct experimental support.

Speciation genes

In animal species, especially *Drosophila*, hybrid sterility is thought to be caused by incompatibility between different species' gene products, rather than by physical incompatibilities between their chromosomes of the types described above. Genetic (or genic) incompatibilities that sterilize (or kill) hybrids are called 'speciation genes' (Wu and Ting, 2004). In principle, yeast F1 hybrid sterility could be caused by genetic incompatibilities that disrupt some aspect of gamete production such as meiosis or sporulation, or those that affect the gametes only after they are formed (Figure 2). Speciation genes that affect F1 hybrid diploids directly should be called dominant (because they are heterozygous with the compatible allele), whereas those that act only in haploid gametes (that is, when hemizygous) are recessive.

To determine whether dominant speciation genes cause F1 hybrid sterility, diploid hybrids between *S. cerevisiae* and the five other *sensu stricto* species were made tetraploid (Greig *et al*., 2002a). This genome doubling meant that all chromosomes received an identical homologue with which to recombine during meiosis, removing the sterilizing effect of rearrangement and antirecombination, but not the effect of any dominant genetic incompatibilites. Fertility was restored to normal in all five tetraploid hybrids. Dominant speciation genes should be unaffected by ploidy, suggesting that diploid hybrids, like tetraploid hybrids, lack dominant sterilizing incompatibilities. But a dominant genetic incompatibility that caused meiotic chromosome non-disjunction might have less sterilizing power in a tetraploid hybrid because the (diploid) gametes produced from a tetraploid meiosis would be more tolerant of aneuploidy than the haploid gametes produced from a diploid meiosis. This possibility remains to be tested.

The observed F1 hybrid sterility in yeast could be caused by recessive speciation genes that kill haploid hybrid gametes or prevent them from dividing mitotically (stages 4 and 5, Figure 2). The rare gametes that survive a hybrid meiosis contain chromosomes from both species, so clearly some combinations of chromosomes are viable (Hunter *et al*., 1996; Greig *et al*., 2002b). But aneuploidy in these gametes might mask recessive incompatibilities—in other words, the hybrid gametes might simply survive because they contain a complete haploid set of one species' chromosomes, as well as some extra chromosomes from the other species. To make euploid hybrid gametes, individual chromosomes in *S. cerevisiae* haploids were replaced with their homologues from *S. paradoxus* (Greig, 2007). Nine of the sixteen *S. paradoxus* chromosomes have been tested to date, and all nine were found to be compatible with the *S. cerevisiae* genome, producing nine viable euploid gametic strains, each containing 15 *S. cerevisiae* chromosomes and a single *S. paradoxus* chromosome.

Recent experiments have shown that these partially hybrid haploids can, as well as dividing by mitosis, also

germinate from spores (D Greig, C Maclean, P Malakasi, unpublished data). Thus none of the nine chromosomes appear to carry speciation genes that cause the observed F1 hybrid sterility. There may be sterilizing speciation genes yet to be found, either because they only work in combination with those on other chromosomes, or because they lurk on the seven as yet untested chromosomes. Nevertheless, it is clear that genetic incompatibility is insufficient to explain the sterility of *S. cerevisiae* × *S. paradoxus* F1 hybrids (Greig, 2007). Ongoing work is aimed at transferring the remaining seven chromosomes, as well as determining the effect of the substituted chromosomes on mating, mating-type switching and F2 homozygous diploid mitosis and meiosis.

The apparent absence of strong genetic incompatibilities between yeast species is surprising. A recent laboratory experiment shows that mild dominant incompatibilities can evolve quite rapidly in yeast (Dettman *et al*., 2007). Initially-identical populations were allowed to evolve for just 500 asexual generations in one of two novel environments. Hybrids made between strains that had adapted to different environments were found to grow less well than non-hybrids, showing that dominant incompatibilities reducing F1 fitness had evolved. The hybrids also had reduced sporulation rates, but normal meiotic fertility. The authors speculated that, given more time, complete genetic incompatibility—hybrid inviability—would evolve. There is a striking contrast between these experimentally evolved incipient species, in which a few mutations have resulted in partial reproductive isolation by dominant genetic incompatibilities causing hybrid inviability (or low viability) but not sterility, and *Saccharomyces sensu stricto* species, which remain largely genetically compatible despite massive sequence divergence causing hybrid sterility but not hybrid inviability. One explanation is that *Saccharomyces* species genomes, while diverse at the sequence level, are functionally conserved, having evolved in much more similar environments than the novel environments imposed experimentally. This is, perhaps, reflected by the fact that *S. paradoxus* and *S. cerevisiae* can be found in exactly the same habitat.

Conclusions

Two forms of reproductive barriers have been identified between *Saccharomyces sensu stricto* species. Premating reproductive isolation in the form of mate choice can reduce hybridization rates, but hybrids still form readily when the nearest available mate is another species. The likely cause of this relatively weak barrier is differences in the timing of spore germination and mating, presumably caused by underlying genetic differences between the species. Postmating reproductive isolation in the form of hybrid sterility is stronger, causing most gametes produced by F1 hybrids to be inviable and fail to form colonies in a laboratory fertility assay. The sterility of F1 hybrids between *S. paradoxus* and *S. cariocanus* is likely caused solely by chromosomal rearrangements, and the sterility of F1 hybrids formed between species with collinear genomes is likely caused solely by antirecombination. Two experiments could confirm these assertions: engineering the genome of *S. cariocanus* to be collinear with *S. paradoxus*, and inducing meiotic crossovers between the diverged chromosomes in *S. cerevisiae* and *S. paradoxus* hybrids. Both factors contribute to the sterility of the other hybrids, but antirecombination is the major factor, because there are not enough chromosomal rearrangements to have a large sterilizing effect. To date, there is no evidence that incompatibilities between the genes of different yeast species contribute to the inability of F1 hybrid gametes to form colonies.

While F1 hybrid sterility is the strongest and most easily detected barrier between yeast species, it is likely that other barriers contribute to reproductive isolation. We do not know enough about the ecology and natural history of wild yeast, especially species other than *S. cerevisiae* and *S. paradoxus*, to know how often species interact. I am not aware of any systematic studies comparing the viability (that is mitotic growth rate, stress resistance and survival) of F1 hybrids with their parent species. It is also likely that other incompatibilities could affect stages of the yeast life cycle beyond F1 hybrid meiosis (Figure 2), and studying the viability and fertility of euploid F2 hybrids could be very informative. But while some similarities with animal and plant reproductive barriers may become apparent in the future, it is clear that the major cause of reproductive isolation in yeast is antirecombination.

Is antirecombination important in other species barriers, or is yeast a special case? One of the most intriguing features of *Saccharomyces*, compared with higher organisms, is the existence of the tetrad ascus, a structure whose function seems to be to promote automixis and to prevent outcrossing (Reuter *et al*., 2007; Coluccio and Neiman, 2004); Johnson *et al*., 2004; Ruderfer *et al*., 2006). If yeast outcrosses only rarely, gene flow between species, though it is known to occur (Liti *et al*., 2006), might be so unlikely an event that it should best be treated like horizontal gene transfer between bacteria. This would explain how sufficient sequence divergence could accumulate for the effect of antirecombination be observed in laboratory hybrids, though it would make antirecombination a laboratory artefact rather than a contributor to the species barrier in natural yeast populations. Indeed, the applicability of the biological species concept to a sexual eukaryote with a bacteria-like breeding system might have to be reassessed. Nevertheless, the study of yeast hybrids will remain a powerful way to examine the interaction between diverged gene products. What is clear is that a much better understanding of yeast natural history and ecology is essential, not only to answer these questions of yeast speciation, but to know in general the evolutionary context in which to place our best genetic model organism.

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