Correspondence

Breaking a species barrier by enabling hybrid recombination

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Hybrid sterility maintains reproductive isolation between species by preventing them from exchanging genetic material[1]. Anti-recombination can contribute to hybrid sterility when different species' chromosome sequences are too diverged to cross over efficiently during hybrid meiosis, resulting in chromosome mis-segregation and aneuploidy. The genome sequences of the yeasts *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* have diverged by about 12% and their hybrids are sexually sterile: nearly all of their gametes are aneuploid and inviable. Previous methods to increase hybrid yeast fertility have targeted the anti-recombination machinery by enhancing meiotic crossing over. However, these methods also have counteracting detrimental effects on gamete viability due to increased mutagenesis[2] and ectopic recombination[3]. Therefore, the role of anti-recombination has not been fully revealed, and it is often dismissed as a minor player in speciation[1]. By repressing two genes, *SGS1* and *MSH2*, specifically during meiosis whilst maintaining their mitotic expression, we were able to increase hybrid fertility 70-fold, to the level of non-hybrid crosses, confirming that anti-recombination is the principal cause of hybrid sterility. Breaking this species barrier allows us to generate, for the first time, viable euploid gametes containing recombinant hybrid genomes from these two highly diverged parent species.

Species are formed and maintained by the restriction of gene flow between diverging populations. Barriers to gene flow can be physical, such as geographic distance, or they can be properties of the species themselves. Here, we focus on one such barrier to gene flow, hybrid sterility. Hybrid sterility can be caused by a variety of mechanisms that can generally be classified into two categories: incompatibilities between diverged chromosomes (such as large-scale chromosomal rearrangements and anti-recombination) and incompatibilities between individual genes from the diverging populations[1].

Here we show that repressing anti-recombination dissolves the reproductive barrier between two yeast species, *S. cerevisiae* and *S. paradoxus*, increasing their production of viable hybrid gametes by 70-fold (Figure 1A). We did this by repressing the meiotic expression of just two highly conserved genes, *SGS1* and *MSH2*. Msh2 is a component of the mismatch repair system that removes base-pair mismatches in duplex DNA, both to repair misincorporations in newly synthesized DNA and to inhibit recombination between diverged sequences (anti-recombination). The former activity reduces mutations, and the latter can help maintain genome integrity by limiting ectopic recombination between non-homologous chromosomes and dispersed repeats[3]. Sgs1 is a DNA helicase that is assumed to act downstream of mismatch recognition by Msh2 to unwind nascent recombination intermediates containing a high density of mismatches[4], but also plays a more general function in recombination to disassemble joint-molecule intermediates that could lead to crossovers[5]. Thus, although completely deleting *MSH2* enhances meiotic recombination between the diverged chromosomes of *S. cerevisiae* x *S. paradoxus* hybrids, increasing proper chromosome segregation and therefore hybrid spore viability[2], this benefit is countered by elevated mutagenesis and genome instability in mitotically dividing cells, reducing viability. We therefore replaced the native promoters of *MSH2* and *SGS1* with the *CLB2* promoter, which represses gene expression during meiosis but not mitosis[6].

Meiotic repression of either gene alone significantly increased hybrid spore viability (Figure 1A, *MSH2* $p = 7.99 \times 10^{-6}$; *SGS1* $p < 2.2 \times 10^{-16}$). Overall spore viability rose from 0.46%

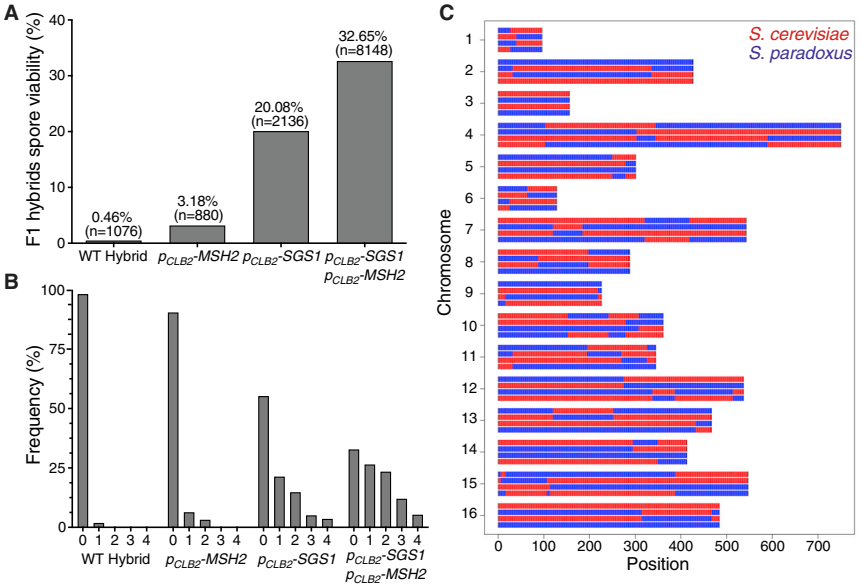

Figure 1. Restoration of hybrid fertility by meiotic repression of *MSH2* and *SGS1*.
(A) Percentages are spore viabilities of the indicated hybrid strains. Both single mutants and the double mutant have significant increases in spore viability. Numbers in parentheses indicate the total number of dissected spores checked for viability. (B) Meiotic repression of *SGS1* and *MSH2* increases the frequency of four-spore tetrads. Full data, including other strains, can be found in the associated Dryad package (Supplemental Information). (C) An example recombination map of a single tetrad. Gametes were genotyped by ORF into one of the two species, ensuring a 2:2 segregation of species identity at each ORF. Only ORFs that were shared and collinear between the two species were considered. Segments are coloured according to their parent of origin.

in the wild-type hybrid to 3.18% in the *pCLB2-MSH2* strain and to 20.08% in the *pCLB2-SGS1* strain. Spore viability was further improved to 32.65% when both genes were repressed ($p < 2.2 \times 10^{-16}$). Although hybrid fertility was not increased to the level of the parents — the *S. cerevisiae* and *S. paradoxus* parent fertilities were 83.75% and 92.25%, respectively — it was well within the range of fertilities of non-hybrid crosses formed from diverged populations of one species or the other. For example, 32–87% for *S. paradoxus* or *S. cerevisiae* crosses with collinear genomes[7], 14–86% for wild *S. paradoxus* crosses[8,9]. These results show that anti-recombination determines most of the hybrid sterility barrier between our *S. cerevisiae* and *S. paradoxus* strains.

This remarkable restoration of hybrid fertility allowed us to produce a large sample of perfectly euploid hybrid gametes. Any viable gametes produced by a hybrid are usually aneuploid[2], and this remains the case even when *MSH2* is knocked out[10]. By dramatically improving hybrid fertility, we significantly increased the production of hybrid tetrads in which all four spores were viable from 0% in the wild-type hybrid to 5.3% in the double mutant hybrid (Figure 1B; 0 out of 269 versus 108 out of 2,037, respectively, $p = 2.04 \times 10^{-4}$). Because all chromosomes are essential in yeast, we can infer that these full tetrads contain only euploid hybrid gametes. Generation of these hybrids enables the unambiguous analysis of recombination and trait mapping, both of which were previously confounded by aneuploidy in sampled hybrid spores[10].

Finally, in order to map the genome-wide distribution of crossovers in our *pCLB2-MSH2 pCLB2-SGS1* double mutant hybrid, we sequenced the genomes of the 336 hybrid spores from 84 fully viable tetrads. We found that crossing over was much increased compared to wild-type hybrid strains with an average of 18.9 crossovers per spore. Figure S1A shows that recombination in our manipulated hybrid followed the normal, non-hybrid pattern of a higher crossover density (cM/kbp) on smaller chromosomes than larger chromosomes (*S. cerevisiae* slope = –0.00013, $p = 0.0032$; hybrid mutant slope = -4.22×10^{-5}, $p = 0.048$), in contrast to the wild type, unmanipulated hybrid measured by Kao *et al.*[10] (slope = -3.82×10^{-8}, $p = 0.99$). Sequencing the genomes of our recombinant hybrid spores revealed that the suppression of anti-recombination activity was evenly distributed across the genomes of both species, rather than being locally enriched at hotspot regions (Figure S1A).

This study shows that repressing the meiotic expression of just two genes, *SGS1* and *MSH2*, overcomes the anti-recombination barrier between two yeast species, restoring the fertility of their hybrids to intraspecific levels, and allowing them to produce viable, euploid, recombinant gametes (Figures 1C and S1B). We demonstrate directly that anti-recombination is the major cause of post-zygotic reproductive isolation between these species. By enabling recombination between such diverged species, our method can be used to identify any intrinsic genetic incompatibilities, or speciation genes, to map the genetics underlying diverged phenotypes, or to produce recombinant hybrids with novel properties for commercial or research use.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure, experimental procedures, author contributions, and supplemental references and can be found with this article online at https://doi.org/10.1016/j.cub.2020.12.038.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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