Epistasis and hybrid sterility in *Saccharomyces*

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Hybrid sterility is thought to be due to deleterious epistatic interactions between genes from different species. Here we demonstrate that dominant genic incompatibility does not contribute to sterility in hybrids between *Saccharomyces cerevisiae* and five closely related species. Sterile diploids were made fertile by genome doubling to produce hybrid tetraploids. Based on these and previous results, we conclude that neither genic incompatibility nor classical chromosomal speciation models apply.

Keywords: speciation; tetraploid; meiosis; yeast; evolution

1. INTRODUCTION

The genetic basis of postzygotic reproductive isolation has become clearer over the last decade. In animals (especially *Drosophila*), genic incompatibility is thought to be the primary cause of hybrid inviability and sterility, as predicted by Dobzhansky (1936, 1937) and Muller (1940, 1942). In plants, postzygotic isolation usually involves karyotypic changes by chromosomal rearrangement (Rieseberg *et al.* 1995), polyploidy (Ramsey & Schemske 1998), or hybridization (Comai 2000). Recent theoretical studies have attempted to determine the evolutionary causes driving postzygotic isolation, both in analyses of the 'Dobzhansky–Muller' (D–M) model of speciation (Turelli & Orr 1995, 2000; Orr & Turelli 2001; Turelli *et al.* 2001) and in speciation associated with karyotypic changes (Rieseberg 2001).

Despite these developments, the genetic basis underlying postzygotic isolation is still not known for most organisms, including the model eukaryote *Saccharomyces cerevisiae* and its five close relatives comprising the *Saccharomyces sensu stricto* complex (Naumov *et al.* 2000). These six *Saccharomyces* species are postzygotically isolated: interspecies F₁ hybrids are viable but sterile, producing only about 1% viable gametes that are generally highly aneuploid. Classical chromosomal speciation cannot be the cause of hybrid sterility, because all *Saccharomyces sensu stricto* species have 16 chromosomes with a total of just four, two or zero rearrangements relative to *S. cerevisiae* that have no correlation with genetic distance (Fischer *et al.* 2000).

In this paper, we test the D–M model in *Saccharomyces* species by assessing the extent of the reproductive isolation due to epistatic interactions. In the D–M model, isolated populations fix different beneficial alleles at different loci, which results in reproductive isolation if the alleles are incompatible when in the same genetic background. In the most simple form, a two-allele two-locus model, genotypes AAbb and aaBB have normal fitness but hybrid genotypes such as aabb have reduced viability or

fertility, as the a and b alleles at the two loci are incompatible. The extent of incompatibility can vary with the 'dominance' of the effect, in that genotypes aabb, Aabb and AaBb may differ in epistatic interactions and hence incompatibility (Turelli & Orr 2000).

Our test of dominant genic incompatibility is the degree of sterility observed in hybrid tetraploids relative to hybrid diploids. *Saccharomyces* species usually live as single-celled diploids that may undergo meiosis when starved, forming a tetrad of four haploid gametes (spores). There are two haploid mating types, a and α , and each set of four spores consists of two of each type. Usually gametes are fertilized upon germination by an opposite mating-type gamete, but, if unfertilized, a haploid can divide mitotically producing a daughter cell. Wild-type gametes can switch mating type after division and the resulting gametes can fertilize each other, restoring fertility (Herskowitz *et al.* 1992). Switching occurs by programmed non-reciprocal recombination replacing the active allele at *MAT* with the allele of the opposite type from silent donor loci (*HMRA* and *HML α*) located elsewhere on the same chromosome (Herskowitz *et al.* 1992). We deleted the *S. cerevisiae* copy of the *MAT* locus in F₁ hybrid diploids. Diploids with two active copies of the *MAT* locus do not have mating type, but with only one copy of *MAT* active, a diploid behaves as a gamete that can divide, switch mating type and auto-fertilize, producing an allo-tetraploid.

2. MATERIAL AND METHODS

(a) Strains

All diploid strains (table 1) are homothallic *HO* diploids. Uncrossed genotypes are homozygous for the indicated auxotrophic markers that were produced by standard yeast techniques (Rose *et al.* 1990). Crosses were performed with YDG 199 and the resulting five F₁ hybrids and one non-hybrid control were selected on minimal medium supplemented with uracil (table 1).

(b) Production of tetraploids

Strains YDG 205, 203, 202, 204, 201 and 222 were transformed with the *PvuII* fragment of plasmid pFP18 (a gift from Frederique Pacques & James Haber). This contains a functional

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Table 1. Species and strain designations used in this study.

strain designation	species and genotype
YDG 186	<i>Saccharomyces bayanus</i> (CBS700123 ^a); <i>ura3</i> , <i>lys2</i> ^b
YDG 196	<i>Saccharomyces cariocanus</i> (UFRJ 50816 ^c); <i>ura3</i> , <i>lys2</i> ^b
YDG 190	<i>Saccharomyces kudriazevii</i> (IFO 1802 ^d); <i>ura3</i> , <i>lys2</i> ^b
YDG 193	<i>Saccharomyces mikatae</i> (IFO1815 ^d); <i>ura3</i> , <i>lys2</i> ^b
YDG 197	<i>Saccharomyces paradoxus</i> (ATCC 16981; N17 ^e); <i>ura3</i> , <i>lys2</i> ^b
YDG 221	<i>Saccharomyces cerevisiae</i> (Y55 ^f); <i>ura3</i> , <i>lys2</i> ^b
YDG 199	<i>Saccharomyces cerevisiae</i> (Y55 ^f); <i>ura3</i> , <i>ade2</i> ^b
YDG 205	YDG 186 × YDG 199, F ₁ diploid hybrid ^b
YDG 203	YDG 196 × YDG 199, F ₁ diploid hybrid ^b
YDG 202	YDG 190 × YDG 199, F ₁ diploid hybrid ^b
YDG 204	YDG 193 × YDG 199, F ₁ diploid hybrid ^b
YDG 201	YDG 197 × YDG 199, F ₁ diploid hybrid ^b
YDG 222	YDG 221 × YDG 199, <i>Saccharomyces cerevisiae</i> diploid ^b

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^b This study.

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^e American Type Culture Collection, Manassas, VA, USA.

^f McCusker & Haber (1988).

URA3 gene flanked by the sequences X, W and Z2 of the mating-type region. The fragment integrates into chromosome III, replacing the entire active *MAT* locus, including the *HO*-endonuclease target sequence. The resulting transformants behave like gametes. Transformants were selected and grown on synthetic media lacking uracil, allowing *HO*-induced mating-type switching to occur within the chromosome lacking the integration. The resulting diploids of the opposite mating type can fertilize the original diploid transformants, producing tetraploids. Transformants were screened for successful tetraploid formation by microscopic examination after induction of meiosis and sporulation. Tetrads from tetraploids are characteristically large compared with the tetrads from diploids.

(c) Measurement of fertility

Strains were plated on sporulation medium and incubated at 25 °C for one week to induce meiosis and tetrad formation. Tetrads were scraped into 50 µl of a 5% solution of glusulase and then 1 ml of sterile water was added immediately. Incubation in this solution for 1 h at room temperature removes the outer wall of the ascospore, allowing the four spores to be separated easily by micromanipulation and placed individually onto the surface of a YEPD (yeast extract 1%, peptone 2%, dextrose 2%) agar plate. We incubated these plates for 3 days at 30 °C and scored as viable spores that germinated and formed a visible colony. Fertility is the number of viable spores out of the total number dissected (or four times the number of tetrads dissected). We measured the fertility of the six non-hybrid species, the five hybrid diploids, three independent tetraploids for each of the five hybrids and two independently transformed tetraploids from YDG 222, the *S. cerevisiae* autotetraploid control.

3. RESULTS

(a) Non-hybrid diploids are fertile

Crosses within species resulted in high spore viability, ranging from ca. 88–99% across the six species (table 2). Spore viability in the laboratory environment varied with species (Kruskal–Wallis test: $p < 0.001$, d.f. = 5).

(b) Hybrid fertility restored in tetraploids

Fertility of diploid hybrids was very low, with spore viability ranging from 0.0–0.83% (table 2), demonstrating the postzygotic isolation among the *Saccharomyces* species, consistent with the species definitions (Naumov *et al.* 2000), and was not correlated (d.f. = 3, $p > 0.5$) with the genetic relatedness nor with the number of translocations (Fischer *et al.* 2000). By contrast, spore viability of the hybrid tetraploids (table 3) was far greater than that of the hybrid diploids in each respective cross (two-tailed Mann–Whitney *U*-tests: $p < 0.001$ for each cross) (see also Banno & Kaneko 1989), even after correcting for performing five simultaneous tests, one for each species pair (Sokal & Rohlf 1995). More importantly, overall hybrid tetraploid fertility was greater than that for the hybrid diploids (two-tailed Mann–Whitney *U*-test: $p < 0.01$), but was indistinguishable from the non-hybrid diploids by both non-parametric (two-tailed Mann–Whitney *U*-test: $p > 0.20$) and parametric (two-tailed *t*-test: $p > 0.30$, d.f. = 8) statistical tests.

There was significant variation in spore viability among the tetraploid hybrids (Kruskal–Wallis test: $p < 0.001$, d.f. = 4), but the variation showed no statistically significant relationship with fertility of the non-*S. cerevisiae* parental species ($r = -0.27$, d.f. = 3, $p > 0.2$; Kendall's $\tau = 0$, $p > 0.2$). A possible explanation for differences in tetraploid fertility is that quadrivalents or trivalents may form instead of bivalents causing problems with meiotic segregation (Loidl 1995). This would be less likely to occur in allotetraploids between species with more diverged chromosomes and, indeed, the tetraploid hybrids between *S. cerevisiae* and the most genetically distant species, *S. bayanus*, do have the highest tetraploid fertility (99%). However, there is no overall correlation between allotetraploid fertility and genetic relatedness (d.f. = 3, $p > 0.4$), possibly because genetic distance is estimated only from ITS (internal transcribed spacer regions of the ribosomal RNA genes) divergence (Fischer *et al.* 2000), whereas overall chromosomal sequence similarity is probably the important factor in determining meiotic pairing.

Table 2. Fertility of *Saccharomyces* species.

species	ploidy	frequency of spore viability per tetrad					fertility (%)
		4	3	2	1	0	
<i>Saccharomyces cerevisiae</i>	2n	95	5	0	0	0	98.75
<i>Saccharomyces bayanus</i>	2n	53	7	0	0	0	97.08
<i>Saccharomyces cariocanus</i>	2n	52	3	3	0	2	92.92
<i>Saccharomyces kudriazevii</i>	2n	37	12	7	0	0	88.39
<i>Saccharomyces mikatae</i>	2n	52	5	0	0	1	96.12
<i>Saccharomyces paradoxus</i>	2n	51	4	4	0	0	94.92

Table 3. Fertility of diploid and tetraploid hybrids.

cross	ploidy	frequency of spore viability per tetrad					fertility (%)	t_s^a	p^b
		4	3	2	1	0			
<i>Saccharomyces cerevisiae</i> × <i>Saccharomyces cerevisiae</i>	2n	95	5	0	0	0	98.75	4.10	<0.001
	4n	28	11	1	0	0	91.88		
<i>Saccharomyces bayanus</i> × <i>Saccharomyces cerevisiae</i>	2n	0	0	0	1	59	0.42	11.59	<0.001
	4n	172	7	0	0	0	99.02		
<i>Saccharomyces cariocanus</i> × <i>Saccharomyces cerevisiae</i>	2n	0	0	0	0	60	0.00	11.49	<0.001
	4n	119	29	21	5	1	87.14		
<i>Saccharomyces kudriazevii</i> × <i>Saccharomyces cerevisiae</i>	2n	0	0	1	0	59	0.83	11.49	<0.001
	4n	159	11	5	1	1	96.05		
<i>Saccharomyces mikatae</i> × <i>Saccharomyces cerevisiae</i>	2n	0	0	0	0	60	0.00	11.24	<0.001
	4n	75	41	35	15	4	74.71		
<i>Saccharomyces paradoxus</i> × <i>Saccharomyces cerevisiae</i>	2n	0	0	0	2	58	0.83	11.57	<0.001
	4n	125	45	6	2	0	91.15		

^a Statistic calculated to evaluate significance of Mann–Whitney *U*-test (Sokal & Rohlf 1995, p. 430) to be compared with $t_{\alpha,4}$.

^b Probability that the difference between diploid and haploid fertilities occurred by chance for a two-tailed test.

4. DISCUSSION

There are several theoretical models of the speciation process (Turelli *et al.* 2001) but no single model applies universally (Dobzhansky 1970; Grant & Grant 1997; Wu 2000). In species with postzygotic isolation, two models have received most experimental support: the evolution of genic incompatibilities between allopatric populations as described by Dobzhansky (1937) and Muller (1942), and the evolution of gross karyotypic changes that disrupt hybrid meiosis (White 1978). Although there are exceptions, experiments with animals typically demonstrate the first mechanism while the second is generally supported by plant studies. The significance of this taxonomic distinction has been unclear and there has been little evidence to signify whether either mechanism applies to other organisms such as fungi or microbes.

We are investigating the cause of reproductive isolation between the model organism *S. cerevisiae* and its five closely related species. Previously, we excluded gross chromosomal changes as a possible cause, as all six species have the same chromosome number and the relatively small number of rearrangements (0, 2, or 4) cannot explain hybrid sterility (Fischer *et al.* 2000). In addition, we have demonstrated that the mismatch repair system contributes to *Saccharomyces* hybrid sterility (Chambers *et al.* 1996; Hunter *et al.* 1996) and may cause speciation (Greig *et al.* (2002), discussed below). In this paper, we exclude dominant genic incompatibilities, one form of

epistasis that could potentially result in speciation by the D–M model.

(a) Dobzhansky–Muller speciation model

Dobzhansky (1933) was the first to assess the importance of dominant genic incompatibilities in speciation, noting that sterility in a *Drosophila* hybrid was due to ‘a profound disturbance of spermatogenesis, including failure of chromosome pairing at meiosis’ (p. 397). He performed an elegant test to distinguish between two possible explanations: either it was caused by ‘chromosomes of one species or race finding no complete homologues among the chromosomes of another species or race’ (p. 402) or it was ‘due to the action of complementary genetic factors contributed by parent species’ (p. 402). He found that in islands of tetraploid spermatocytes chromosomes still failed to pair, just as in normal diploid spermatocytes. He therefore argued that the sterility could not be due to the former explanation, because in tetraploid cells ‘the doubling of the chromosome complement furnishes an exact homologue to every chromosome’ (p. 402) (figure 1). He concluded that the sterility was caused by genic incompatibilities that exist whether the hybrid cells examined are diploid or tetraploid.

The basis of the D–M model is that epistatic interactions between genes cause sterility or inviability in F_1 hybrids or subsequent F_2 or backcross hybrids. Effects in the F_1 generation are due to dominant epistatic interactions,

Figure 1. Chromosome segregation during meiosis. (a) In a normal diploid each parental chromosome replicates, pairs and forms crossovers with its homologue. At the first meiotic division (I), homologues segregate into different cells. The second meiotic division (II) is a mitosis-like separation of chromatids at the centromere. (b) In a hybrid, recombination and segregation fail because the parental homologous chromosomes are not alike. (c) In a tetraploid produced by a doubling of chromosomes each chromosome can segregate from an identical partner.

because F_1 hybrids are heterozygous for all alleles. In F_2 or backcross hybrids, loci may be homozygous for incompatible alleles, allowing for recessive genic incompatibility and hybrid breakdown. Here, we performed Dobzhansky's tetraploid test on yeast hybrids and, in contrast to Dobzhansky's result, we observed a dramatic restoration of fertility. This rescue of *Saccharomyces* hybrids by doubling chromosome number demonstrates that dominant epistasis did not contribute to reproductive isolation.

Recessive epistasis is a factor in reproductive isolation of other species. Incomplete dominance has been demonstrated by crosses of *Xiphophorus maculatus* (platyfish) with *X. helleri* (swordtails) in which lethal tumours cause death (Dobzhansky 1937; see also Orr & Presgraves 2000). F_1 hybrids are viable although they develop benign melanomas, but one-quarter of the $F_1 \times X. helleri$ backcross generation develop malignant melanomas, one-quarter develop benign melanomas and one-half are healthy. This hybrid breakdown is the result of a two-locus system, a sex-linked tumour locus and an autosomal suppressor locus. More evidence for the importance of recessive incompatibilities has been obtained in many *Drosophila* studies, particularly as an explanation of Haldane's rule (Haldane 1922), which states that if hybrid inviability or sterility is sex-specific then it occurs predominantly in the heterogametic sex (Presgraves & Orr 1998; Turelli & Orr 2000). Yeast gametes express their genes, therefore recessive genic incompatibilities could be exposed in haploid gametes produced by F_1 hybrids, as well as in F_2 or backcross hybrids. Our current study does not address the role that recessive incompatibilities might play in hybrid sterility, which may vary with taxa and mechanism of sex determination, as discussed below.

(b) Different mechanisms for different taxa

In plants, postzygotic reproductive isolation usually has a chromosomal basis, whereas D–M-mediated speciation is thought to be more important in animals. This difference may be because the genome is expressed in pollen, but not in sperm, so gross karyotypic changes are more likely to cause gamete inviability in plants than in animals

(Turelli & Orr 2000; Rieseberg 2001). Also, most plants are either hermaphroditic or have only minimally degenerate Y-chromosomes, unlike most animals. Degeneracy of the Y-chromosome allows X-linked, partially recessive genes to evolve quickly and thus generate genic incompatibilities between animal species (Rieseberg 2001). This is thought to be an underlying cause of Haldane's rule. Another possible explanation is that animal development, being tightly programmed, is more easily disrupted by a randomly evolving genic incompatibility than the less sensitive development of plants.

Saccharomyces species are more similar to plants than animals in that genes are expressed in gametes, there are no sex chromosomes and development is even less constrained than for plants, as the most complex structure that yeast makes is the four-celled tetrad. However, unlike plants, postzygotic isolation is not due to chromosomal rearrangements (Fischer *et al.* 2000), but surprisingly, neither does it appear to be due to genic interactions as in the D–M model.

Two mechanisms of reproductive isolation consistent with our results are loss or silencing of duplicated genes and mismatch repair. Different copies of duplicated genes can be lost or silenced among species. Gametes produced by hybrids may contain only null or silenced alleles for a particular duplicated gene, causing sterility (Werth & Windham 1991; Lynch & Force 2000). Tetraploidy could allow recovery of fertility because the gametes of allotetraploids are expected to contain complete sets of both species' chromosomes. Our results provide no evidence either for or against this hypothesis and this mechanism has yet to be tested. To date, the only mechanism of reproductive isolation in *Saccharomyces* species that has empirical support is mismatch repair-mediated genome incompatibility. Inactivation of the mismatch repair system (by gene deletion) increases fertility 10-fold in hybrids between *S. cerevisiae* and *S. paradoxus* (Hunter *et al.* 1996). In addition, the mismatch repair system is responsible for ca. 50% of the postzygotic reproductive isolation between diverged *S. cerevisiae* strains Y55 and YP1, and a similar amount between allopatric populations of *S. paradoxus*

(Greig *et al.* 2002). The mismatch repair system detects nucleotide mismatches, preventing successful recombination between diverged sequences and causing faulty chromosome segregation and hybrid sterility. Therefore, general sequence divergence is probably the major cause of F_1 hybrid sterility in yeast.

(c) Conclusion

Speciation of the six species in the *Saccharomyces sensu stricto* complex does not appear to have occurred by either of the two common models of postzygotic isolation. The absence of dominant genic incompatibilities and of factors associated with recessive genic incompatibilities (e.g. chromosomal sex determination) indicates that speciation did not evolve via the D–M model. Karyotypic changes have evolved but are limited to chromosomal rearrangements that are few in number and are not correlated with fertility. Previous results (Chambers *et al.* 1996; Hunter *et al.* 1996; Greig *et al.* 2002) indicate that the mismatch repair system has mediated a novel mode of speciation in *Saccharomyces*. Thus, current models of postzygotic reproductive isolation do not apply to all organisms.

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