Report

Chromosomal Rearrangements as a Major Mechanism in the Onset of Reproductive Isolation in *Saccharomyces cerevisiae*

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Summary

Understanding the molecular basis of how reproductive isolation evolves between individuals from the same species offers valuable insight into patterns of genetic differentiation as well as the onset of speciation [1, 2]. The yeast *Saccharomyces cerevisiae* constitutes an ideal model partly due to its vast ecological range, high level of genetic diversity [3–6], and laboratory-amendable sexual reproduction. Between *S. cerevisiae* and its sibling species in the *Saccharomyces* sensu stricto complex, reproductive isolation acts postzygotically and could be attributed to chromosomal rearrangements [7], cytonuclear incompatibility [8, 9], and antirecombination [10, 11], although the implication of these mechanisms at the incipient stage of speciation remains unclear due to further divergence in the nascent species. Recently, several studies assessed the onset of intraspecific reproductive isolation in *S. cerevisiae* by evaluating the effect of the mismatch repair system [12–14] or by fostering incipient speciation using the same initial genetic background [15–18]. Nevertheless, the overall genetic diversity within this species was largely overlooked, and no systematic evaluation has been performed. Here, we carried out the first species-wide survey for postzygotic reproductive isolation in *S. cerevisiae*. We crossed 60 natural isolates sampled from diverse niches with the reference strain S288c and identified 16 cases of reproductive isolation with reduced offspring viabilities ranging from 44% to 86%. Using different mapping strategies, we identified reciprocal translocations in a large fraction of all isolates surveyed, indicating that large-scale chromosomal rearrangements might play a major role in the onset of reproductive isolation in this species.

Results and Discussion

To obtain a global view of the landscape of intraspecific reproductive isolation in *Saccharomyces cerevisiae*, we selected 60 natural isolates from diverse ecological and geographical niches (Table S1 available online). Estimated genetic divergence within these strains ranges from 0.11% to 0.60%, which is a relatively comprehensive representation of the genetic diversity currently observed in this species (Figure 1). We crossed all isolates with the reference strain S288c and estimated the offspring viability for each cross. A relatively large fraction of crosses (16 out of 60) qualified as cases of reproductive isolation, with reduced offspring viabilities ranging from 44% to 86% (Table S1). No apparent

correlation was observed between the estimated genetic divergence of the parental pairs and the resulting offspring viability (Figure 2), indicating that general DNA sequence differences were not sufficient to explain the observed reproductive isolation.

To understand the molecular basis and complexity underlying the identified cases, additional tetrads were dissected for all 16 incompatible crosses (Table S2), and the segregation of the lethal phenotype was analyzed (Figure S1). Six cases showed mild reduction of offspring viability (78%–87%, mean = 82%; 65 tetrads analyzed on average) (Figure 2; Table S2), which resulted in a Poisson distribution with decreasing number of full tetrads (four viable spores, Figure S1). This segregation pattern suggests these cases were probably caused by a mutator [13, 14] or antirecombination [12] effect of the mismatch repair system, as previously observed. The remaining 10 cases with a higher degree of progeny loss (44%–74%) were further analyzed.

Bulk Segregant Analysis Revealed a Unique Reciprocal Translocation Responsible for Cases of Reduced Offspring Viability of ∼75%

According to the segregation, eight crosses (between S288c and DBVPG1339, DBVPG4651, M22, T73, Y9J, L-1528, YJM978, and YJM981) showed predominantly three types of tetrads with four, three, or two viable spores (Figure 1; Figure S1). The ratio between these tetrad types was roughly 1:2:1, resulting in a reduced spore viability of ∼75% (66%–74%, mean = 71%; 228 tetrads analyzed on average) (Table S2). In addition, pairwise crosses among all eight strains showed offspring viabilities higher than 90% (data not shown), indicating these cases represented a unique genetic origin. To map the genomic regions involved, we focused on one cross (between DBVPG1339 and S288c) and performed bulk segregant analysis by sequencing a pool of 50 independent segregants from tetrads with only two viable spores, where the lethal genotype combination was absent (Figure S2A). Following this selection, genomic regions involved were expected to have allele frequencies skewed from 0.5, whereas the rest of the genome should have equal proportions of alleles from each parent.

Two regions with significantly skewed allele frequencies were mapped (Figure 3; Figure S3). The first one was located at the left-arm region of chromosome VIII and the second near the centromeric region of chromosome XVI (Figure 3). Additionally, the end of chromosome VIII (∼15 kb) showed a low coverage (∼30×), whereas the left arm of chromosome XVI (∼370 kb) showed a coverage that was nearly 200×, indicating that two copies of the left arm of chromosome XVI might be present (Figure 3). This unbalanced inheritance suggests the presence of a reciprocal translocation between chromosome VIII and chromosome XVI. In fact, when crossing strains bearing the translocation with the reference strain S288c, offspring would inherit either a balanced or unbalanced set of chromosomes (Figure S2B) [19]. Because the region involved on chromosome VIII was near the telomere and does not contain any essential genes, only unbalanced spores with two copies of the left arm of chromosome XVI were
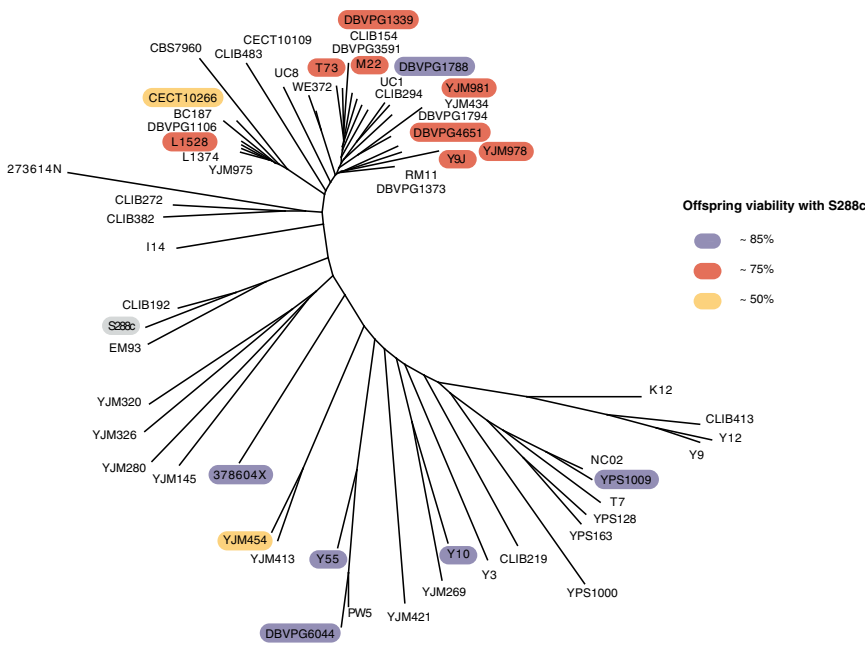
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Figure 1. Neighbor-Joining Tree of 60 Studied *S. cerevisiae* Isolates

A majority-rule consensus tree of the surveyed strains was built based on the 101,343 segregating sites identified in [3]. Branch lengths are proportional to the number of segregating sites that differentiate each pair of strains. Isolates that are incompatible were color coded according to the offspring viability resulting from the cross with the reference S288c. See also Tables S1 and S2 and Figure S1 for detailed strain origins and phenotype segregations for the incompatible crosses.

viable, as was evident by the abnormal coverage. PCR results demonstrated in all eight strains that the translocation occurred between the promoter region of *ECM34* (YHL043W) on chromosome VIII and the promoter region of *SSU1* (YPL092C) on chromosome XVI (Figure 4). Analysis of the junctions revealed no significant homology, suggesting that the translocation originated via a nonhomologous end-joining event.

Successive Backcross Strategy Identified Multiple Reciprocal Translocations Responsible for the Reduced Offspring Viability of ∼50%

The remaining two crosses—CECT10266 and YJM454 with S288c—showed a reduced spore viability of 50% (44%–48%, mean = 46%; 100 tetrads analyzed on average) (Table S2); where three major types of tetrads were observed, each contained four, two, or zero viable spores with a ratio of 1:2:1 (Figure S1). Based on the segregation pattern, we reasoned that the most plausible explanation was the presence of a reciprocal translocation involving two large chromosomal regions, each of which contains at least one essential gene [19]. In this context, any meiotic recombination will lead to missegregation of essential genes and consequently only the progeny that inherited a balanced set of chromosomes would be viable (Figure S2B). Moreover, the cross between CECT10266 and YJM454 demonstrated a further reduction of offspring viability (∼25%; data not shown), indicating that these two strains probably underwent different events leading to the observed reproductive isolation.

Because in these cases all viable F1 segregants would have an equal probability of inheriting either balanced parental genome, no allele frequency variation would be observed by pooling the F1 segregants. We then developed a strategy based on successive backcrossing and next-generation sequencing to map the regions involved. F1 segregants that maintained the phenotype of 50% offspring viability were successively backcrossed to S288c for five generations, in order to obtain a single segregant enriched for the S288c

genome but still retaining the original translocation. Each fifth generation backcross segregant, namely, CS-B5 (from the cross between CECT10266 and S288c) and YS-B5 (from the cross between YJM454 and S288c), was subjected to whole-genome sequencing. Due to limited recombination around the junctions, the genomes of these backcrossed segregants would be otherwise allelic to S288c, except for regions involved in the translocation.

Identification of a Reciprocal Translocation between Chromosomes VII and XII in CECT10266

Genome sequencing of the segregant CS-B5 (from the cross between CECT10266 and S288c) revealed two regions that are polymorphic to S288c. The first region was approximately located on the left arm of chromosome VII and the second on the right arm of chromosome XII (Figure S4A). Breakpoints of the putative translocation were identified using PCR. The first breakpoint was located between *MCM6* (YGL200C) and *EMP24* (YGL201C) on chromosome VII and the second between YLR326W and *NMA1* (YLR328W) on chromosome XII. Considering the relative position of the centromeres on these two chromosomes, the translocation likely occurred between the left arm of chromosome VII and the right arm of chromosome XII (Figure 4), leading to two new chimeric chromosomes with functional centromeres. The junctions of this putative translocation were confirmed using PCR. Sequencing of the amplified fragments revealed a full-length Ty2 transposon at both junctions (Figure 4), suggesting that the translocation likely originated from homologous recombination (HR) between Ty elements.

Identification of a Reciprocal Translocation between Chromosomes V and XIV in YJM454

Similarly, we also mapped two regions in the genome of YS-B5 (from the cross between YJM454 and S288c). The first region was found on the right arm of chromosome V and the second region on the left arm of chromosome XIV (Figure S4B). Based on the same principle, we identified two breakpoints: the first located between *PMD1* (YER132C) and *GLC7* (YER133W) on chromosome V and the second between *PHO23* (YNL097C) and *RPS7B* (YNL096C) on chromosome XIV. In this case the right arm of chromosome V was likely exchanged with the left arm of chromosome XIV to ensure centromeric functions of the chimeric chromosomes (Figure 4). Indeed, PCRs confirmed the presence of both junctions involved in this
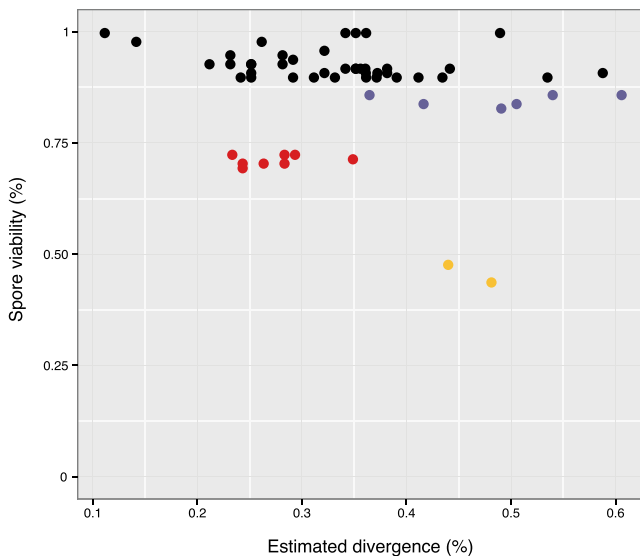
Figure 2. Sequence Divergence Does Not Correlate with the Observed Offspring Viability

The estimated sequence divergence between each pair of parental strains (horizontal axis) was plotted against the observed offspring viability (vertical axis). All strains were crossed with the reference strain S288c, and the offspring viability was estimated by dissecting 20 tetrads. Crosses with offspring viabilities <90% were color coded. Blue: crosses with offspring viability of ~85%. Red, crosses with offspring viability of ~75%; yellow, crosses with offspring viability of ~50%.

putative translocation (Figure 4). Sequence analysis of the junctions revealed a full-length Ty2 transposon at both junctions and an additional 3 kb fragment containing a partial Ty4 element at the junction uniting the right arms of chromosomes V and XIV (Figure 4). The presence of multiple Ty elements suggests that the breakpoints might overlap with potential Ty insertion hot spots. This translocation was probably also mediated by HR through Ty elements.

Relative Importance of Chromosomal Rearrangements in Yeast Speciation

The process of speciation is often quantitative, because the strength of reproductive isolation varies continuously at different levels of divergence [2]. The yeast *S. cerevisiae* and its close relatives in the *Saccharomyces* sensu strico complex offer a unique opportunity to explore the possible mechanisms leading to the onset of intrinsic reproductive isolation at both "short" (within species) and "long" (between species) evolutionary scales.

Including *S. cerevisiae*, six species are currently circumscribed in this group [20], all of which readily cross with each other to form viable hybrids [21]. Yet, interspecific hybrids showed strong postzygotic reproductive isolation, producing only ~1% of viable offspring [21, 22]. Many species in this group differ by chromosomal rearrangements [7, 22, 23]; however, because this only partially explains the substantial loss of hybrid progeny due to the extant high interspecific divergence, the relative role of translocations in the onset of reproductive isolation and speciation in these species was largely debated [7, 24, 25].

In this study, we found that chromosomal rearrangements, especially reciprocal translocations, play a substantial role in the onset of reproductive isolation in *S. cerevisiae*. The

fact that this type of mechanism exists at different temporal levels of genetic divergence, both within and between species, suggests that reciprocal translocations might have a larger impact to the onset of speciation in yeast than previously thought.

Adaptation through Chromosomal Rearrangements Is Common in *S. cerevisiae*

Chromosomal rearrangements, including polyploidies, aneuploidies, segmental duplications, and translocations, are frequently observed in wild and domesticated strains of *S. cerevisiae* [26–29], and such rearrangements could readily be associated with adaptation to environmental stress. For example, the translocation between chromosomes VIII and XVI observed in this study was previously identified in several wine strains, conferring to an advantageous sulfite-resistant phenotype, as this compound was commonly used in wine making [30, 31]. Interestingly, among the eight strains identified here, only five were associated with wine (T73, Y9J, L-1528, M22, and DBVPG1339), whereas the others were from various niches, including clinical sources (YJM978 and YJM981) and white truffle (DBVPG4651) (Figure 1; Table S1), suggesting this translocation was dispersed and might have been selectively maintained across different populations.

In fact adaptive chromosomal rearrangements were frequently observed on different spatiotemporal scales, both in nature [32] and in short-term laboratory evolution [33–35]. These observations, in agreement with our data, suggest that chromosomal rearrangements might offer a mechanism of rapid response to stress and become fixed in the population, despite the potential loss of offspring.

Do Dobzhansky-Müller Incompatibilities Exist in Yeast?

In theory the Dobzhansky-Müller model of genetic incompatibility offers the inherent link between divergent adaptation and reproductive isolation. If two populations evolved to adapt to different environments, mutations accumulated independently in each specialized group may cause negative interactions that reduce hybrid fitness or viability [36]. To date, few pairs of "Dobzhansky-Müller genes" have been identified in plants, insects, and animals, both among and within species [1, 37–41]. Curiously, between different yeast species, genetic incompatibilities appear to be scarce, and hardly any examples have been described [42–44].

Moreover, by screening a large collection of ecologically diverse strains of *S. cerevisiae*, we found no classic Dobzhansky-Müller gene pairs, which would generally affect 25%–50% of the offspring depending on the dominance or recessivity of the genes involved. The only cases found that could implicate genic interactions are from the 85% spore viability class. Yet the segregation pattern of these cases (Figure S1) strongly suggests a "mutator" or "antirecombinogenic" phenotype [12–14] and not a classic two-gene interaction model. Overall, these observations suggest that the classic Dobzhansky-Müller genetic incompatibility scenario is probably rare and might have a modest effect in the onset of postzygotic reproductive isolation in this species.

The lack of awareness concerning such incompatibilities in yeast might be due to the incomplete penetrance of antagonic genetic interactions on permissive rich media. Future research should explore the possibility of incompatibilities related to different conditions such as temperature, media composition, or exposure to various chemical compounds in order to obtain a more complete picture of the molecular mechanisms
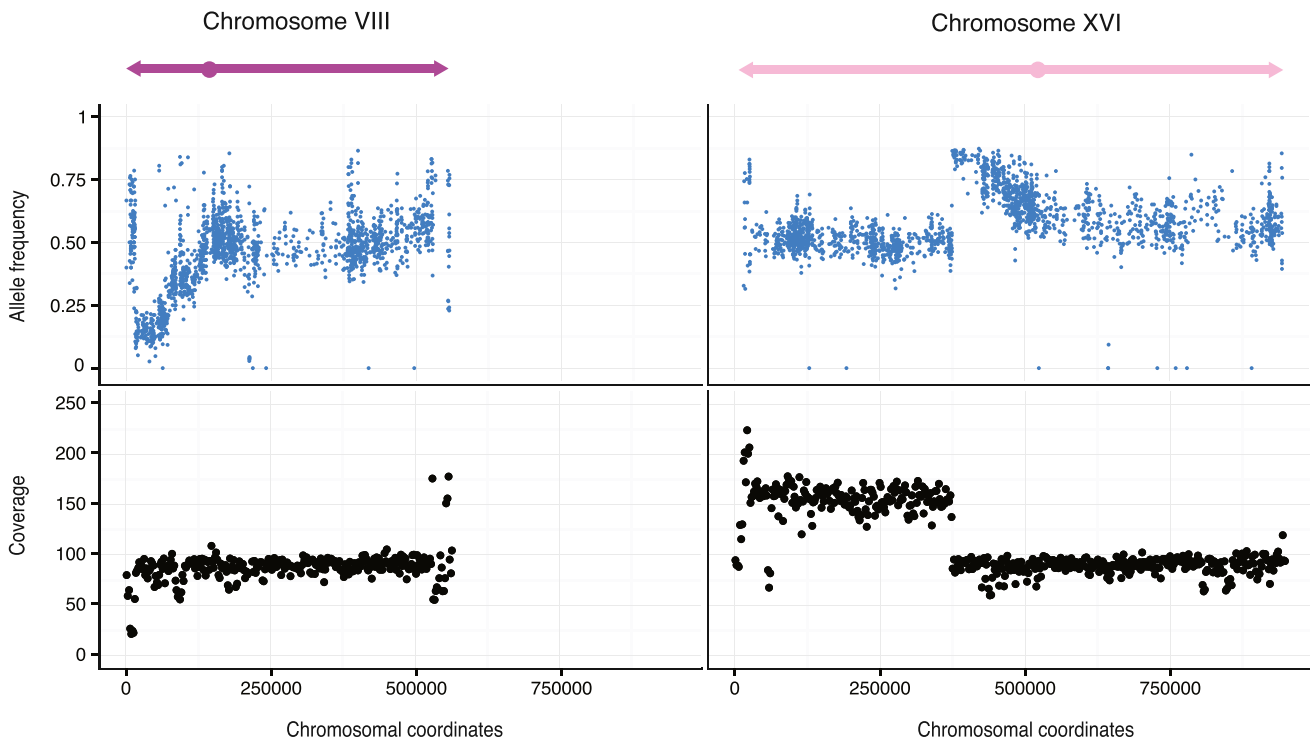
Figure 3. Bulk Segregant Analysis Mapped Two Regions with Skewed Allele Frequencies and Abnormal Coverage

Plot was obtained using bulk segregant data from cross between DBVPG1339 and S288c. The horizontal axis represents the coordinates of chromosomes VIII and XVI. The upper vertical axis corresponds to the allele frequencies of S288c: values close to 1 imply that only alleles of S288c are present and vice versa. The lower vertical axis represents the sequencing coverage in a 2 kb window. The theoretical coverage was expected to be 100× if a single copy was present. Two regions showed significant allele frequency variations: the left-arm region of chromosome VIII (position 15,000–71,000) and the centromeric region of chromosome XVI (position 374,000–453,000). See also Figure S3 for the complete mapping results.

involved in the onset of intraspecific reproductive isolation in *S. cerevisiae*.

Experimental Procedures

Strains
A collection of 60 strains isolated from diverse ecological (tree exudates, wine, different fermentations, and clinical sources) and geographical (Europe, Asia, Africa, and America) origins were used in this study (Table S1). Laboratory strains isogenic to S288c, FY4 (*MAT*a), and FY5 (*MAT*α) were also used.

Media and Culture Conditions
Yeast cells were grown on YPD media (1% yeast extract, 2% peptone, and 2% glucose) using liquid culture or solid plates. Crosses were carried out on YPD plates by mixing freshly grown cells with the opposite mating type. Sporulation was induced on potassium acetate plates (1% potassium acetate and 2% agar). All procedures were done at 30°C.

Test of Spore Viability
All strains screened in this study are *MAT*α and were systematically crossed to FY4 (*MAT*a). Diploids obtained from different crosses were sporulated, and the spore viability for each cross was scored after tetrad dissection. Tetrad asci were gently digested by zymolyase (MP Biomedicals MT ImmunO 20T) and then dissected using a Singer MSM-400 micromanipulator. Spores were aligned on YPD plate and cultured for 48 hr. Viable spores will form colonies, and the spore viability corresponds to the ratio between the number of viable spores and the total number of spores dissected. The first screening was done by analyzing 20 tetrads for each cross. Additional tetrads were dissected for incompatible crosses as listed in Table S2.

Bulk Segregant Analysis Strategy
For cases with 75% spore viability, the segregation of the lethal phenotype resulted in predominantly three types of tetrads: tetrads with four viable

spores or parental ditypes (PDs), three viable spores or tetratypes (TTs), and two viable spores or nonparental ditypes (NPDs) (Figure S2). To map the genomic regions involved, we used bulk segregant analysis strategy by pooling a set of viable spores from NPD tetrads. The cross between DBVPG1339 and FY4 was selected for the mapping. In total, 300 tetrads were dissected, and 50 independent spores from NPD tetrads were separately cultured and then pooled by equal optical density readings at 600 nm. Regions involved were mapped by analyzing the allele frequency variation along the genome.

Successive Backcrossing Strategy
For both cases with 50% spore viability, only segregants that inherited either parental genotype were viable, resulting in a segregation of predominantly three types of tetrads: PDs with four viable spores, TTs with two viable spores, and NPDs with zero viable spores (Figure S2). To map the genomic regions involved, we used a successive backcrossing strategy. For each cross, i.e., the cross between CECT10266 and S288c and the cross between YJM454 and S288c, one F1 PD tetrad (four viable spores) was selected, and all four spores were backcrossed to S288c with opposite mating types (FY4 or FY5). Spore viabilities were analyzed, and a segregant that retained the 50% spore viability segregation was selected for a subsequent backcross to S288c. Five generations of backcrosses were performed, and one fifth generation backcrossed segregant (B5) was obtained for each cross, namely, CS-B5 for the segregant derived from the cross between CECT10266 and S288c and YS-B5 for the segregant derived from the cross between YJM454 and S288c. Using this strategy, the majority of the genome was enriched for S288c alleles except for regions involved in low spore viability.

DNA Extraction, Sequencing, and SNP Calling
Genomic DNA was extracted using the QIAGEN Genomic-tip kit. Sequencing of the samples was performed using Illumina Hiseq 2000 technology. We used paired-end libraries, 101 bp/read, and 100× coverage for bulk segregants and 50× coverage for backcrossed segregants.
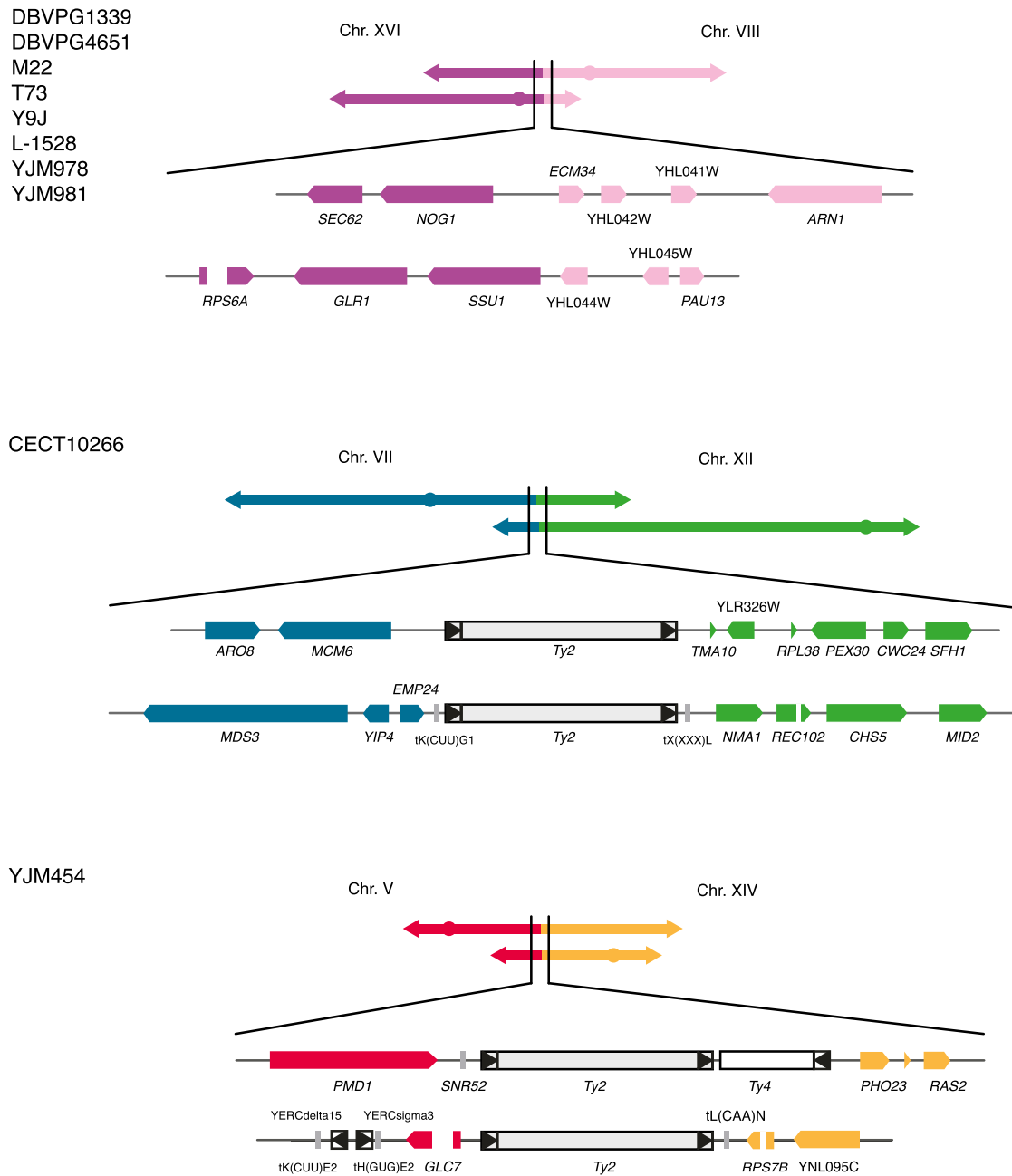
Figure 4. Identified Translocations Responsible for the Observed Reproductive Isolation

Schematics of translocations identified in this study. Chromosome (Chr.) pairs involved are color coded. Chromosome and gene sizes are scaled according to *Saccharomyces* Genome Database annotations. See also Figure S4 for the complete mapping results for the backcrossed segregants.

Quality-controlled reads were aligned to the S288c genome using BWA [45] with "-n 5 -o 2" options. SNP calling was done using SAMtools [46]. The allele frequency of S288c was scored at each polymorphic position. Coverage along the genome was calculated by averaging the number of reads aligned at each genomic position within a 2 kb window.

Neighbor-Joining Tree

A majority-rule consensus tree of the surveyed strains was built based on the 101,343 segregating sites identified by Schacherer et al. [3]. For strains that were not represented in the original tree [3], the publicly available sequences [47] were recovered Yeand aligned against the S288c reference sequence with BWA (-bwasw option), except for the CECT10266 strain, for which we computed our own reads mapping (see DNA Extraction, Sequencing, and SNP Calling above). Polymorphic positions were called with SAMtools and used to complete the segregating sites matrix. We constructed a neighbor-joining tree of the strains studied from these SNP data using the software package Splitstree [48], with branch lengths proportional to the number of segregating sites that differentiate each node.

Supplemental Information

Supplemental Information includes four figures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.03.063.

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