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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 post-zygotically and could be attributed to chromosomal rearrangements [7], cyto-nuclear incompatibility [8, 9] and anti-recombination [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 post-zygotic 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 to the onset of reproductive isolation in this species.

Keywords

intraspecific diversity; reproductive isolation; translocations; yeast

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Results and discussion

To obtain a global view of the landscape of intraspecific reproductive isolation in *S. cerevisiae*, we selected 60 natural isolates from diverse ecological and geographical niches (Table S1). 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). 6 cases showed mild reduction of offspring viability (78% to 87%, mean=82%; 65 tetrads analyzed on average) (Figure 2; Table S2), which resulted in a Poisson distribution with decreasing number of full tetrads (4 viable spores, Figure S1). This segregation pattern suggests these cases were probably caused by a mutator [13, 14] or anti-recombination [12] effect of the mismatch repair system, as previously observed. The remaining 10 cases with a higher degree of progeny loss (44% to 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, 8 crosses (between S288c and DBVPG1339, DBVPG4651, M22, T73, Y9J, L-1528, YJM978 and YJM981) showed predominantly 3 types of tetrads with 4, 3 or 2 viable spores (Figure 1, Figure S1). The ratio between these tetrad types was roughly 1:2:1, resulting in reduced spore viability of ~75% (66% to 74%, mean=71%; 228 tetrads analyzed on average) (Table S2). In addition, pairwise crosses among all 8 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 S2-A). 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 (~30X) whereas the left-arm of chromosome XVI (~370 kb) showed a coverage that was nearly 200X, 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 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 S2-B) [19]. As 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 viable, as was evident by the abnormal coverage. PCR results demonstrated in all 8 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 Non-Homologous End-Joining (NHEJ) event.

Successive backcross strategy identified multiple reciprocal translocations responsible for the reduced offspring viability of ~50%

The remaining 2 crosses (CECT10266 and YJM454 with S288c) showed a reduced spore viability of 50% (44% to 48%, mean=46%; 100 tetrads analyzed on average) (Table S2), where 3 major types of tetrads were observed, each contained 4, 2 or 0 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 mis-segregation of essential genes and consequently only the progeny that inherited a balanced set of chromosomes would be viable (Figure S2-B). 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.

Since 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 5 generations, in order to obtain a single segregant enriched for the S288c genome but still retaining the original translocation. Each 5^{th} 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 chromosome 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 S4-A). 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 chromosome 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 one was found on the right-arm of chromosome V and the second on the left-arm of chromosome XIV (Figure S4-B). 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 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 chromosome V and XIV (Figure 4). The presence of multiple Ty elements suggests that the breakpoints might overlap with potential Ty insertion hotspots. This translocation was probably also mediated by Homologous Recombination (HR) through Ty elements.

Relative importance of chromosomal rearrangements in yeast speciation

The process of speciation is often quantitative, as the strength of reproductive isolation varies continuously at different levels of divergence [2]. The yeast *Saccharomyces 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 post-zygotic reproductive isolation, producing only ~1% of viable offspring [21, 22]. Many species in this group differ by chromosomal rearrangements [7, 22, 23], however, as 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 chromosome 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 8 strains identified here, only 4 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 which reduce hybrid fitness or viability [36]. To date, few pairs of “Dobzhansky-Muller 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 50% to 25% of the offspring depending on the dominance or recessivity of the genes involved. The only cases found which could implicate genic interactions are from the 85% spore viability class. Yet, the segregation pattern of theses cases (Figure S1) strongly suggests a “mutator” or “anti-recombinogenic” 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 post-zygotic 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 involved in the onset of intraspecific reproductive isolation in *S. cerevisiae*.

Experimental procedures

Strains

A collection of 60 strains isolated from diverse ecological (tree exudate, wine, different fermentations and clinical) and geographical (Europe, Asia, Africa and America) origins was 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, 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 (MT ImmunO™ 20T) and then dissected using micromanipulator Singer MSM-400. Spores were aligned on YPD plate and cultured for 48 hours. 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 3 types of tetrads: tetrads with 4 viable spores or parental ditypes (PD), 3 viable spores or tetratypes (TT) and 2 viable spores or non-parental ditypes (NPD) (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 then pooled by equal O.D. 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 which inherited either parental genotypes were viable, resulting in a segregation of predominantly 3 types of tetrads: parental ditypes (PD) with four viable spores, tetratypes (TT) with 2 viable spores, and non-parental ditypes (NPD) with 0 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 parental ditype tetrad (PD, 4 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

which has retained the 50% spore viability segregation was selected for a subsequent backcross to S288c. Five generations of backcrosses were performed and one 5th 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 100X coverage for bulk segregants and 50X coverage for backcrossed segregants. Quality controlled reads were aligned to the S288c genome using BWA with "-n 5 -o 2" options. SNP calling was done using SAMtools [45]. 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. 2009. For strains that were not represented in the original tree [3], the publicly available sequences [46] were recovered and 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* section). Polymorphic positions were called with SAMtools and used to complete the segregating sites matrix. We constructed a neighbour-joining tree of the strains studied from these SNP data using the software package Splitstree [47], with branch lengths proportional to the number of segregating sites that differentiate each node.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- First systematic survey of post-zygotic reproductive isolation in *S. cerevisiae*
- Progeny loss was not correlated with sequence divergence at the intraspecific scale
- Reproductive isolation in *S. cerevisiae* was mainly related to genome rearrangements
- Pervasiveness of such rearrangements suggest an essential role to yeast speciation

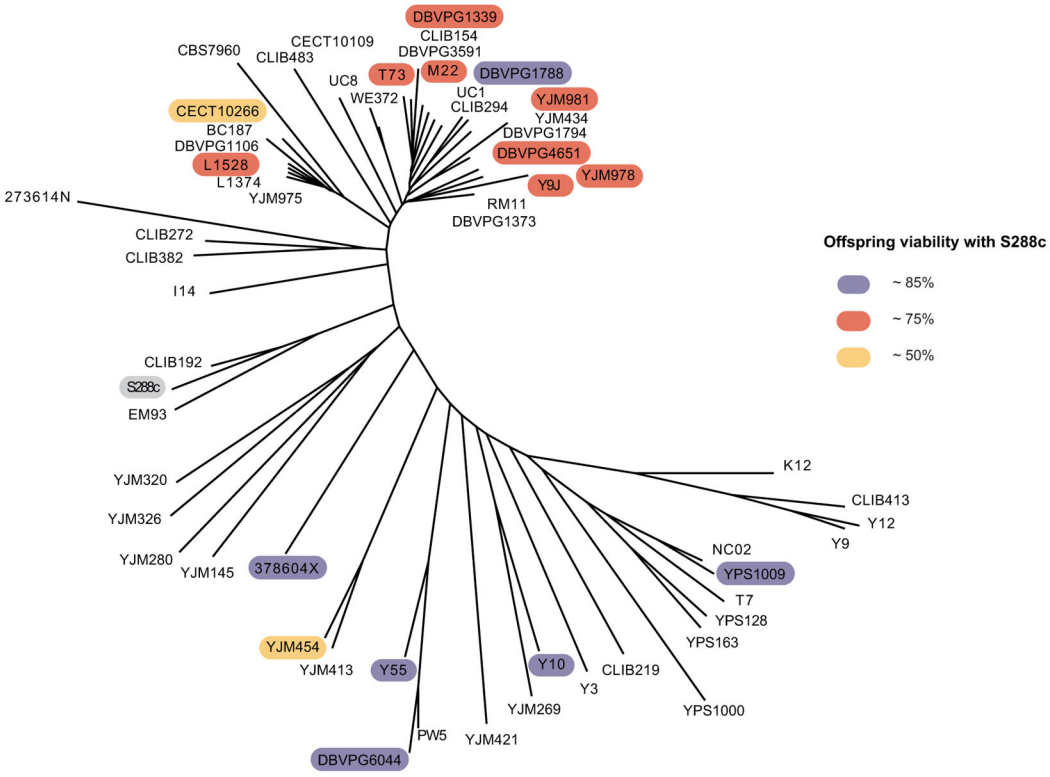

Figure 1. Neighbor-joining tree of 60 studied *S. cerevisiae* isolates

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 Table S1 and Figure S1 for detailed strain origins and phenotype segregations for the incompatible crosses.

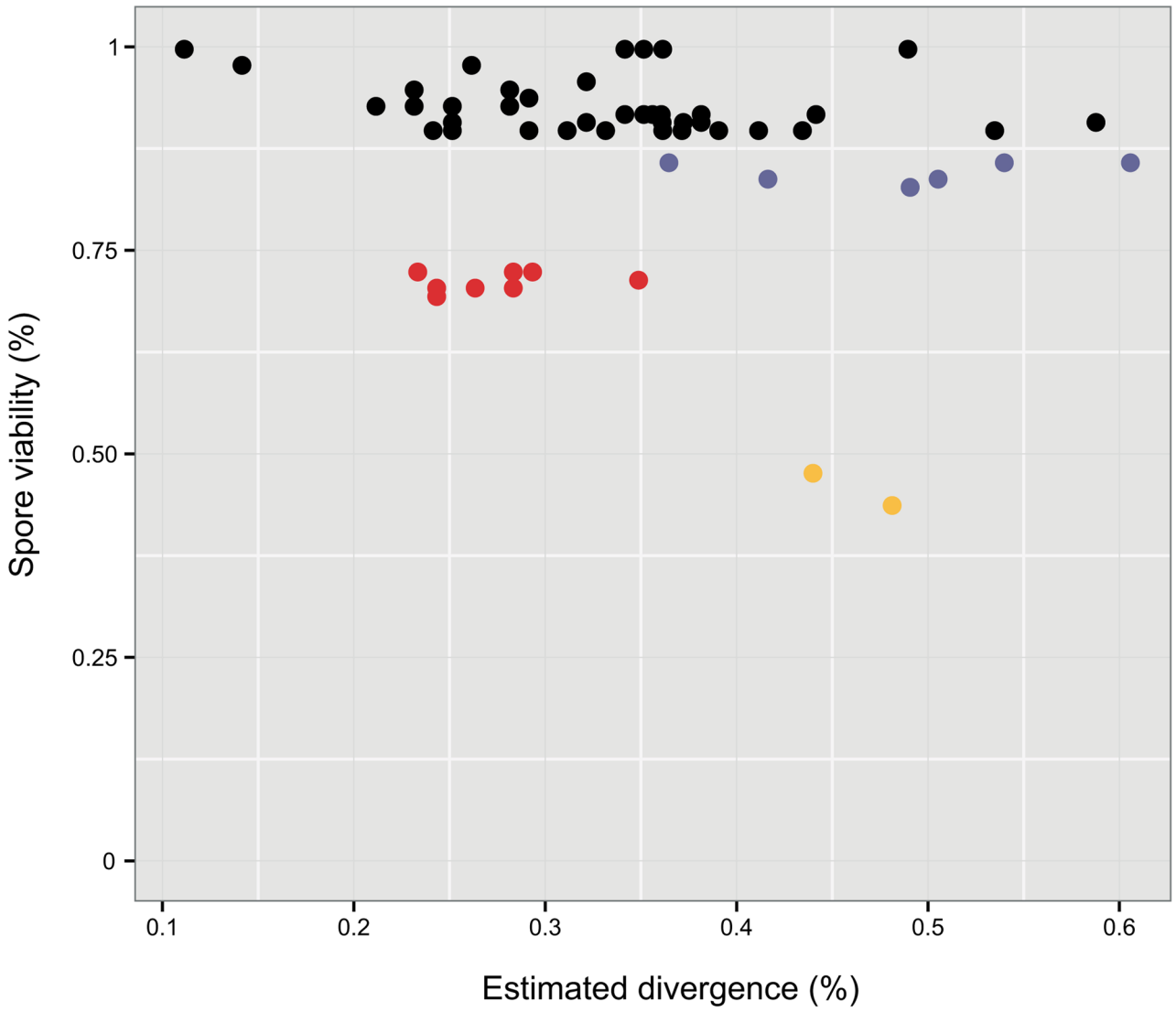

Figure 2. Sequence divergence does not correlate with the observed offspring viability

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%.

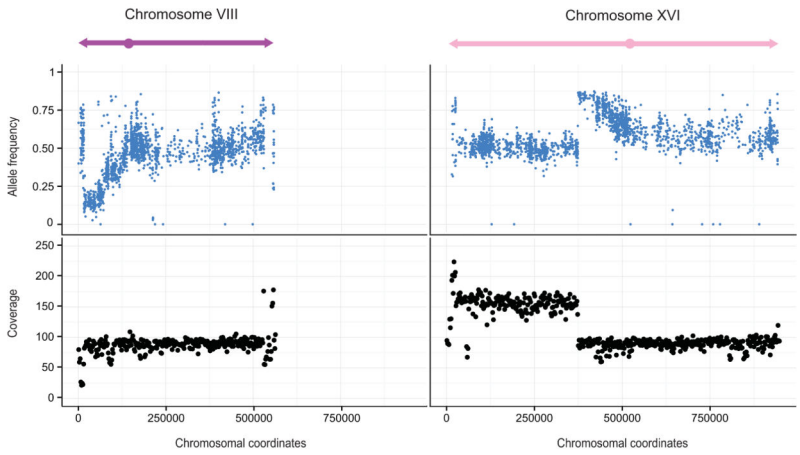

Figure 3. Bulk segregant analysis mapped two regions with skewed allele frequencies and abnormal coverage

Plot was obtained using bulk segregant data from cross between DBVPG1339 and S288c. The horizontal axis represents the coordinates of chromosome 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 100X if a single copy was present. Two regions showed significant allele frequency variations: the left-arm region of chromosome VIII (position 15000 to 71000) and the centromeric region of chromosome XVI (position 374000 to 453000). See also Figure S3 for the complete mapping results.

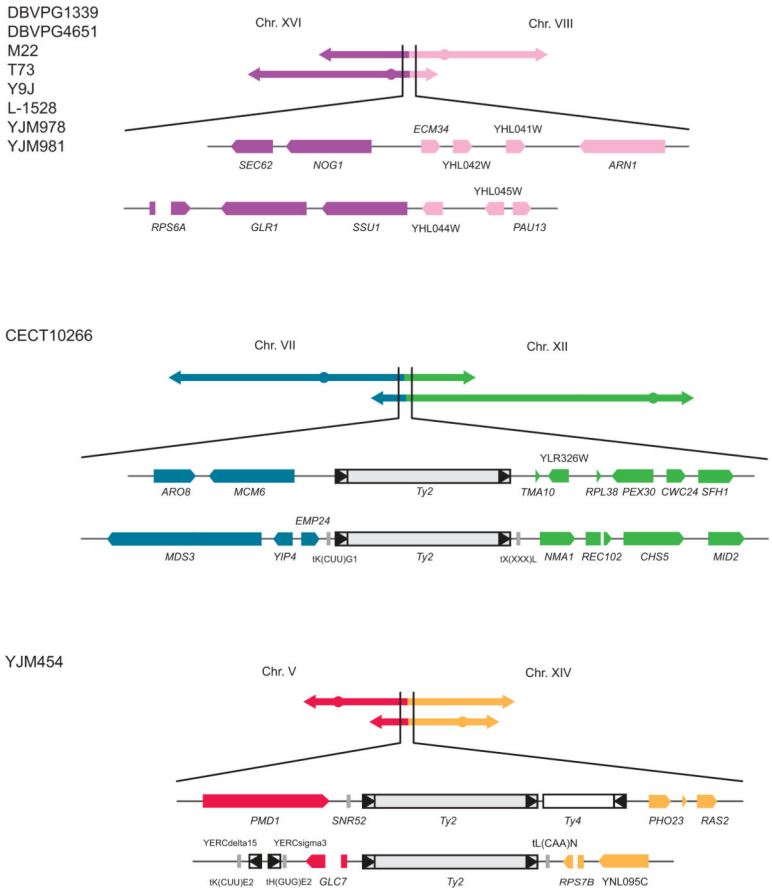

Figure 4. Identified translocations responsible for the observed reproductive isolation

Schematics of translocations identified in this study. Chromosome pairs involved are color-coded. Chromosome and gene sizes are scaled according to SGD annotations. See also Figure S4 for the complete mapping results for the backcrossed segregants.