Effects of reciprocal chromosomal translocations on the fitness of *Saccharomyces cerevisiae*

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Yeast species have undergone extensive genome reorganization in their evolutionary history, including variations in chromosome number and large chromosomal rearrangements, such as translocations. To determine directly the contribution of chromosomal translocations to the whole organism's fitness, we devised a strategy to construct in *Saccharomyces cerevisiae* collinear 'evolutionary mimics' of other species originally differing by the presence of reciprocal translocations in their genome. A modification of the Cre/*lox*P system was used to create in *S. cerevisiae* the translocations detected in the sibling species *Saccharomyces mikatae* IFO 1815 and 1816. Competition experiments under different physiological conditions showed that the translocated strains of *S. cerevisiae* consistently outcompeted the reference *S. cerevisiae* strain with no translocation, both in batch and chemostat culture, especially under glucose limitation. These results indicate that chromosomal translocations in *Saccharomyces* may have an adaptive significance, and lend support to a model of fixation by natural selection of reciprocal translocations in *Saccharomyces* species.
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INTRODUCTION

Nuclear genome rearrangements are common events during the evolutionary history of yeast species. They include variations in chromosome number (Keogh *et al*, 1998; Langkjaer *et al*, 2000; Špírek *et al*, 2003), differences in gene order resulting from duplication events followed by differential gene loss (Llorente *et al*, 2000; Fischer *et al*, 2001), inversions (Huynen *et al*, 2001) and reciprocal translocations (Fischer *et al*, 2000). Owing to their short life cycle and the availability of excellent genetic tools, the *Saccharomyces* 'sensu stricto' species complex represents a good model system to investigate the fitness effects of reciprocal

translocations. Among these species, translocations are not distributed evenly, raising the possibility that they might be adaptive (Fischer *et al*, 2000). The recently isolated yeast species *Saccharomyces mikatae* is characterized by the presence of reciprocal translocations, involving chromosomes VI, VII and XVI, with respect to *Saccharomyces cerevisiae*. The impact of chromosomal translocations on reproductive isolation between these two species has already been reported, showing that restored collinearity can increase hybrid fertility (Delneri *et al*, 2003). In this work, we wanted to investigate the effect of these naturally occurring rearrangements on the whole organism's evolutionary fitness (defined as the probable genetic contribution of an individual or a genotype to succeeding generations).

So far, it has been difficult to assess the fitness effect of translocations in yeast species, owing to the presence of other genetic or epigenetic differences between strains or species. To isolate the specific effects of reciprocal translocations, excluding any other genetic or epigenetic components, we needed to obtain isogenic strains differing exclusively by the presence of translocations. *S. cerevisiae* is the ideal system to investigate this issue, owing to the availability of standard laboratory strains and powerful tools for genetic manipulation. Therefore, we decided to 'mimic' the naturally occurring reciprocal translocations of *S. mikatae* in *S. cerevisiae*. We used a modification of the Cre/*lox*P system to engineer four strains of *S. cerevisiae*: one acting as a reference strain (r-ScWT, no translocations), two mimicking the genome structure of *S. mikatae* IFO 1816 (ScT1, one translocation) and IFO 1815 (ScT1T2, two translocations), and one (ScT2) not collinear with any isolate so far obtained from nature. All strains were isogenic, except for the engineered translocations. In an asexually propagating microorganism, the relative fitness is given by the population growth rates of the different cell types, as they compete for a pool of resources. In our experiments, the relative fitness of the strains was assessed in competition experiments under different nutrient limitations (C-, N-, P- and S-limited media). We showed that ScT1 and ScT1T2 consistently outcompeted the reconstituted wild-type strain (r-ScWT) in batch and chemostat culture, especially under glucose limitation, whereas strain ScT2, in batch culture, did not show any significant growth advantage. Our results indicate that the translocations present in *S. mikatae* have a significant effect on the fitness of *S. cerevisiae* in glucose-limited laboratory conditions. As strains of *S. mikatae* have been isolated from nutritionally poor habitats, such as soil

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Table 1 | Batch competition experiment: intrinsic growth rates (growth per hour between 0 and 24 h) of individual strains in YPD and SD and selection coefficients (strain 1/strain 2 = relative change in frequencies in strain 1 per generation) of pairwise competition

	YPD	SD
Intrinsic growth rates (h^{-1})		
Sc	0.261 (0.006)	0.253 (0.010)
ScT1	0.273 (0.008)	0.276 (0.013)
ScT2	0.265 (0.006)	0.25 (0.009)
ScT1T2	0.266 (0.007)	0.221 (0.006)
Selection coefficients		
Sc/ScT1	−0.229 (0.036)***	0.027 (0.029)
Sc/ScT2	0.013 (0.006)***	0.038 (0.025)**
Sc/ScT1T2	0.005 (0.004)**	0.048 (0.081)
Sct1/ScT1T2	0.11 (0.004)***	0.018 (0.023)
Sct2/ScT1T2	−0.166 (0.099)***	−0.022 (0.03)

The value shown is the average selection coefficient from the five replicates. Standard deviation values are given in parentheses. Selection coefficients significantly different from 0 (*t*-test) are marked (*at the 95% confidence level; **at the 99% confidence level; ***at the 99.9% confidence level).

and decaying leaves (Naumov *et al*, 2000), our data suggest that the translocations found in this species may have conferred some advantage in natural conditions. This is compatible with a model of fixation of chromosomal rearrangements by natural selection.

RESULTS

Construction of strains for the competition experiment

To assess the effect that chromosomal rearrangements have on yeast growth, we constructed four strains differing from one another by specific translocations. *S. cerevisiae* r-ScWT is a strain that has been taken through the complete process for the introduction of translocations. It has a reconstituted genome that is collinear with that of the original wild-type strain, and contains four *loxP* 'scars' at the selected translocation breakpoints (see Fig 1). *S. cerevisiae* ScT1 and ScT1T2 are strains in which translocations have been introduced to render their genomes collinear with those of *S. mikatae* strains 1816 and 1815, respectively. Finally, *S. cerevisiae* strain ScT2 shares one translocation with *S. mikatae* 1815, but otherwise has a genome configuration that has not been found in any *Saccharomyces* species so far isolated from nature.

To discriminate between the effects of the genomic rearrangements and other genetic effects, all the strains used in the competition experiments needed to be isogenic (Fig 1). To achieve this, the Sct1 strain (Delneri *et al*, 2003) underwent two series of transformations to introduce the *loxP–Kan^r–loxP* and *loxP–KlURA3–loxP* cassettes into the translocation breakpoints identified for *S. mikatae* IFO 1815 (between ORFs YGR188c/YGR189c and YPL108w/YPL109c). The resulting strain was transformed with a Cre recombinase plasmid (Delneri *et al*, 2000) and, upon induction of the recombinase, different translocation events occurred at the positions of the *loxP* sites present in the genome. The colonies were screened by PCR for the ScT1, ScT1T2, ScT2

and r-ScWT chromosomal configurations. In this way, all four strains were obtained from the Sct1 strain, had undergone the same transformation process, and all possessed four *loxP* scars at the translocation breakpoints (even if, as in r-ScWT, the chromosomes retained their wild-type configuration). Karyotypes of the newly generated strains were checked by pulse field gel electrophoresis (PFGE).

Batch competition

The intrinsic growth rates of each strain in YPD and SD media were measured, and the selection coefficients in pairwise competition experiments were determined (Table 1). When comparing the intrinsic growth rates of the same strain grown either in YPD or SD media, no major differences were detected, with the exception of ScT1T2, whose growth rate decreased, relative to r-ScWT, in SD medium. When comparing the intrinsic growth rates of different strains in YPD and SD media, ScT1 showed the highest rate of growth in both media. Competition experiments carried out in YPD showed that ScT1 had a significant advantage over both r-ScWT ($s = 0.229$) and ScT1T2 ($s = 0.110$), whereas ScT1T2 outcompeted ScT2 ($s = 0.166$). These results are in agreement with predictions from the intrinsic growth rates for the different strains. On YPD, all strains carrying the translocation T1 showed the largest competitive advantage, regardless of the presence of translocation T2, with similar selection coefficients for the pairs r-ScWQT/ScT1 and ScT2/ScT1T2 (*t*-test, $P = 0.24$, $n = 5$). Conversely, strains carrying translocation T2 showed different selective advantages depending on the presence of translocation T1 (comparison of the pairs r-ScWT/ScT2 and ScT1/ScT1T2, *t*-test, $P < 0.001$, $n = 5$). The effects of each individual translocation do not appear to be additive, as indicated by the comparison of the selection coefficients of the three translocated strains with that of the reconstituted wild type. When competition experiments were carried out in SD medium, no selection coefficient for any pairwise strain comparison showed high values.

Continuous culture competition experiments

The changes in the proportions of the three strains during chemostat competition in four culture conditions are shown in Fig 2A. In all cases, a plateau was reached after 35 generations, after an initial change in the frequencies of the strains. Such plateau effects are not unusual in chemostat culture, and may result from interactions between the strains via the growth medium (Baganz *et al*, 1998; Discussion). The last sample, at 66 generations, showed much higher variation between replicates than the previous one. This could be caused by the occurrence of secondary mutations in some replicates. Therefore, the analysis was performed from the estimated strain frequencies after 53 generations.

Selection coefficients (see Fig 2B) were much lower than in the batch experiment (less than 0.1), but significantly different from zero. The strongest competitive advantage was observed under carbon limitation, and the lowest under sulphate limitation. Under carbon and phosphate limitation, selection favoured ScT1T2 at the expense of r-ScWT, whereas the ScT1 frequency did not change significantly. Under ammonium limitation, ScT1 was favoured, whereas r-ScWT was selected against. Under sulphate limitation, there was a slight but significant advantage for ScT1T2 at the expense of ScT1.
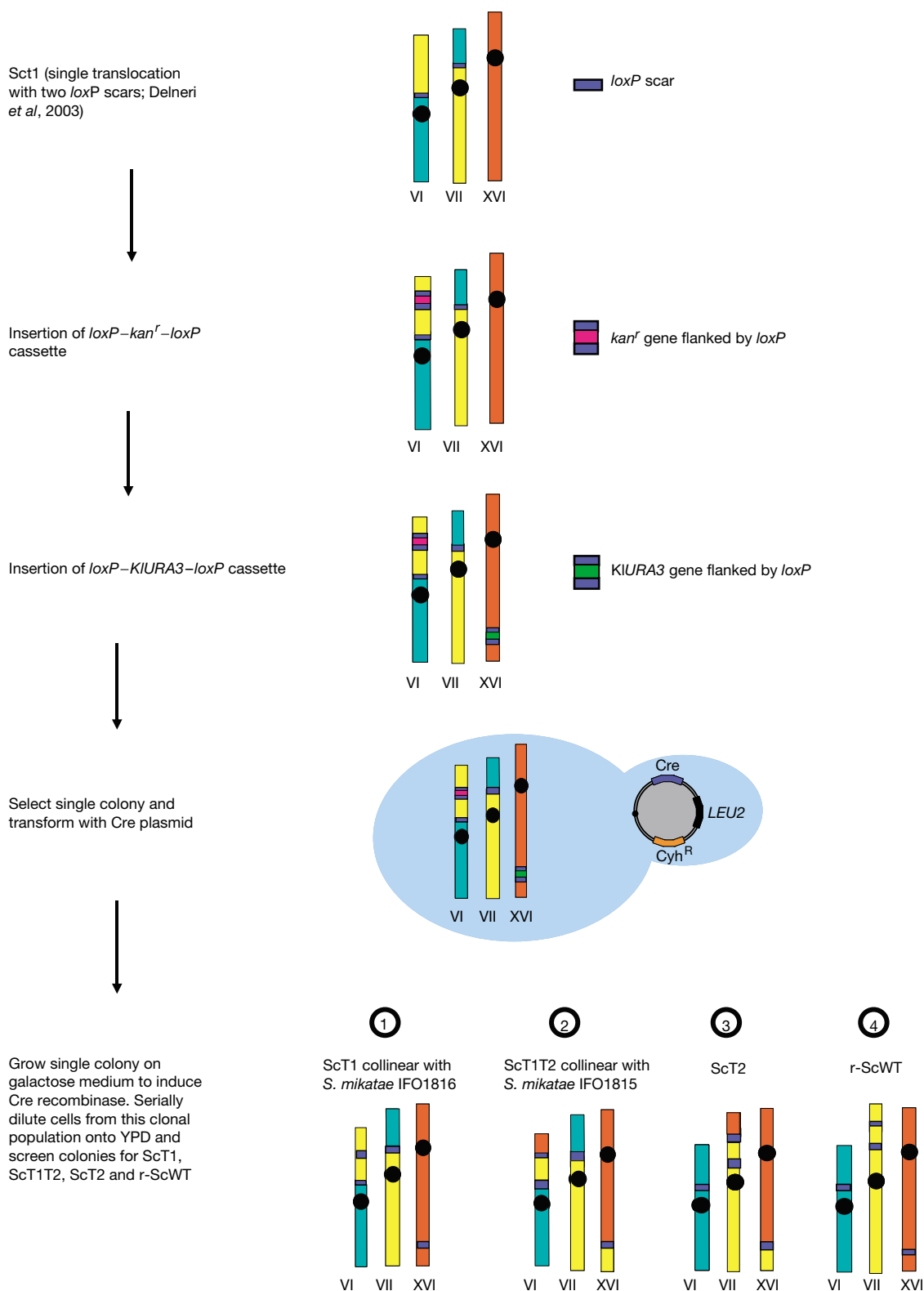
Fig 1 | Strategy adopted to construct isogenic strains differing by chromosomal rearrangements. *loxP–kanr–loxP* and *loxP–KlURA3–loxP* cassettes were inserted in the Sct1 genome at the translocation breakpoints relative to *S. mikatae* IFO 1815. A single colony was selected and transformed with a plasmid containing the Cre recombinase. The transformants were selected on minimal media lacking leucine, and single colonies were transferred onto galactose media to induce the recombinase. Serial dilutions onto YPD were performed and the colonies were screened by PCR for the genotypes ScT1, ScT1T2, ScT2 and r-ScWT.
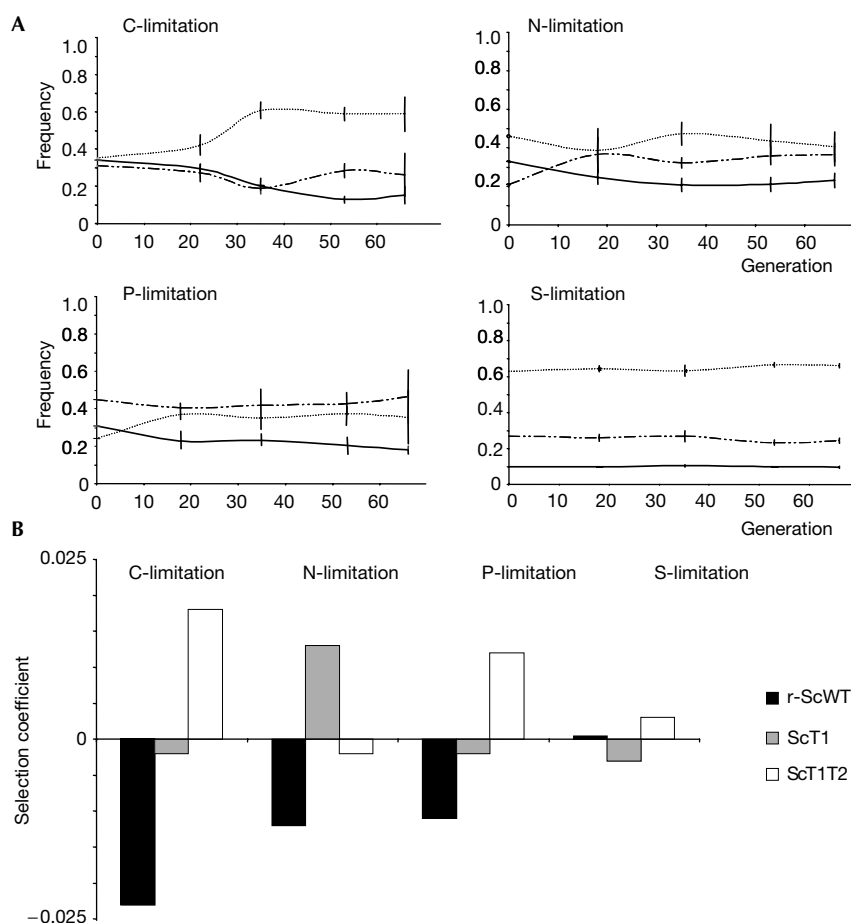
Fig 2 | Selection effects of translocations measured in continuous cultures. (**A**) Changes in the relative proportions of the different strains in the population grown in chemostat culture competition under four nutrient limitations. r-ScWT (—), ScT1 (– · – · –), ScT1T2 (· · · · · · ·). (**B**) Selection coefficients of each strain in the chemostat competition experiment under four different nutrient limitations.

DISCUSSION

S. cerevisiae strains grown in glucose-limited chemostats have been found to show chromosomal rearrangements after approximately 500 generations and, in some cases, to share the same translocation breakpoints. The fact that similar genomic rearrangements recur in different strains suggests that they may be adaptive and responsible for the increased fitness of these strains (Dunham *et al*, 2002). In addition, chromosomal rearrangements could occur during standard laboratory growth conditions (Hughes *et al*, 2000), and have also been shown to be involved in the adaptive evolution of wine yeast strains. A reciprocal translocation between chromosomes VII and XVI appears to cause overexpression of the *SSU1* gene, which makes the wine yeasts resistant to high concentrations of sulphite (Péréz-Ortín *et al*, 2002). In higher eukaryotes, such as *Drosophila*, inversion polymorphisms have also been associated with fitness variation (Fernandez-Iriarte & Hasson, 2000).

All but two of the competition experiments described here (the batch SD experiment, and chemostat under sulphate limitation) provided evidence for positive fitness effects of reciprocal translocations. Even though these effects are modest in the case of chemostat cultures, they are significant. The greatest effect was observed under batch culture in standard YPD medium. In this case, the T1 translocation showed a strong selective advantage ($s > 0.1$), irrespective of the strain's status for the T2 translocation. In contrast, the T2 translocation showed a selective advantage only in strains possessing the T1 translocation. The order of occurrence of these two translocations in *S. mikatae* may be inferred from the species' phylogeny, and is T1 (the sole translocation present in *S. mikatae* 1816 strain) followed by T2 (the *S. mikatae* 1815 strain possesses both translocations). No *S. mikatae* strain so far isolated from nature has been found to contain the T2 translocation on its own. Moreover, T2 involves one of the reciprocal chromosomal products of the T1 event. It could also be postulated that, where multiple translocations have been preserved in natural isolates, there may be some synergistic interactions between them to confer some greater selective advantage than the individual translocations.

Fitness effects of translocation in minimal (SD) medium and in chemostats under nutrient limitation were much smaller than in YPD, with selection coefficients lower than 0.03. Glucose was the principal source of carbon and energy in all of the media. Glucose was chosen as it is the one sugar that is omnipresent in grapes at all stages of the ripening process (Lafon-Lafourcade, 1983).

Although glucose was the main carbon source, population densities were (of course) much higher in the rich YPD medium than in the minimal media. This, together with the plateau effect seen in some chemostat experiments, suggests that the selected strain may alter the growth environment in such a way that the selection conditions are not constant. Such frequency-dependent effects may have important evolutionary consequences (Greig & Travisano, 2004).

Our data indicate that reciprocal translocations in *Saccharomyces* can have positive fitness effects and provide evidence for an increase in maximum specific growth rate in translocated strains of *S. cerevisiae*. At the molecular level, the increased fitness may be the result of changes in the expression of specific genes (as with sulphite resistance in wine yeasts; Péréz-Ortín *et al*, 2002) or changes in the structure of the chromosome themselves (e.g. by the redistribution of replication origins).

It is, of course, difficult to extrapolate our data on a laboratory strain of *S. cerevisiae* to explain the particular evolutionary history of another species. Our results are important in showing that chromosomal rearrangements (and especially those mimicking naturally occurring translocations) have the potential to provide a selective advantage in certain environmental conditions. In this respect, they support a model of chromosomal evolution involving fixation of major genomic rearrangements by natural selection. It cannot be argued that our methodological approach reflects natural conditions, and more research is needed into the natural history of *S. mikatae* to gain insights into the actual evolutionary forces acting in the fixation of chromosomal translocations in that species. If *S. mikatae* were indeed mainly found in sugar-limited habitats, which contrasts to the famous rotting fig from which the progenitor of most laboratory strains of *S. cerevisiae* was isolated, this would give more support to the notion of the adaptive fixation of a favourable translocation.

METHODS

Yeast strains. *S. cerevisiae* Sct1 (Delneri *et al*, 2003) is collinear with *S. mikatae* 1816 and it was derived from *S. cerevisiae* FY23 (*MATa ura3-52 leu2Δ1 trp1Δ63*). The strains ScT1, ScT1T2, ScT2 and r-ScWT were constructed in this work. ScT1 and ScT1T2 are collinear with *S. mikatae* IFO 1816 and 1815 and present, respectively, one and two translocations compared with *S. cerevisiae* wild type. The strain ScT2 differs by one translocation from *S. cerevisiae*, and from both *S. mikatae* strains. r-ScWT is collinear with *S. cerevisiae*, but has *lox*P scars at the translocation breakpoints found in the two *S. mikatae* strains.

All strains were maintained on medium containing 2% yeast extract, 1% peptone and 2% glucose (YPD). To select transformants, cells were grown on YPD–agar containing 300 μg/ml geneticin (Gibco BRL), or on glucose minimal medium (SD) containing 0.67% yeast nitrogen base (Difco), 0.5% ammonium sulphate and 2% glucose (amino acids or nucleic acid bases were added at a concentration of 20 mg/l).

Genetic manipulation. The *lox*P loci were inserted in the genome by PCR-mediated gene replacement (Baudin *et al*, 1993; Wach *et al*, 1994) using either the *kan*ʳ gene or the *KlURA3* gene from *Kluyveromyces lactis*, as selectable markers. Yeast cells were transformed using the lithium acetate method (Gietz *et al*, 1995). Primers flanking the translocation breakpoints on chromosomes VI, VII and XVI were designed, and verification of translocation

events was performed by analytical PCR (Delneri *et al*, 2003). All primers used for engineering and verifying the translocations are listed in Table 2. PFGE was performed according to Fischer *et al* (2000).

Batch competition experiments. Pairs of strains were assessed for competitive ability in YPD and SD media. Five pairs were tested (r-ScWT/ScT1, r-ScWT/ScT2, r-ScWT/ScT1T2, ScT1/ScT1T2, ScT2/ScT1T2), and the growth rate of each of the four strains was determined. All experiments were carried out with five replicates. For the growth assay, 6.25×10^5 cells were inoculated in 5 ml medium. Cell counts were estimated from the OD_{600} after 12, 24, 30, 36 and 48 h. The carrying capacity and intrinsic growth rate were estimated from the cell counts, with carrying capacity $K = N_{48h}$, where N_{48h} is the cell count after 48 h (at stationary phase), and intrinsic growth rate

$$r = \frac{1}{t}\ln\left[\frac{N_t}{N_0}\left(\frac{K - N_0}{K - N_t}\right)\right],$$

where N_0 is the cell count at time 0, N_t is the cell count at time t (24 h) and K is the number of cells at carrying capacity (Mable, 2001). Pairwise competition experiments were conducted in YPD and SD media. In all, 6.25×10^5 cells of each strain were inoculated in 5 ml medium, and were grown for 48 h until the stationary phase. Strain frequencies were estimated by quantitative PCR (see below), and selection coefficient (Dykhuizen, 1993) was calculated as follows: $s = 1/t[\ln(x1_t/x2_t) - \ln(x1_0/x2_0)]$, where t is the number of generations (around eight generations in this experiment), $x1_t$ and $x2_t$ are the frequencies of strains 1 and 2 at time 48 h, and $x1_0$ and $x2_0$ are the frequencies of strains 1 and 2 at the time of inoculation.

Continuous culture competition experiments. Competition experiments were conducted in four different nutrient-limited conditions: glucose-, ammonium-, phosphate- and sulphate-limited (Baganz *et al*, 1998). An equal volume of overnight cultures of three strains (r-ScWT, ScT1 and ScT1T2) was inoculated into a flask containing 100 ml medium and allowed to compete for 66 generations in a Fedbatch-pro (Das Gip Technology) system, at 30 °C, pH 4.5 and shaking at 200 rpm, with a dilution rate of 0.125 h⁻¹. Samples were taken every 4 days (about 18 generations) to assess strain frequencies by quantitative PCR (see below). Each competition experiment was conducted in four replicates. Selection coefficients were determined from the strain frequencies after 53 generations of culture to avoid bias due to uncontrolled mutations. Indeed, mutations with an effect on growth tend to occur every 50 generations (Brown *et al*, 1998; Ferea *et al*, 1999).

Quantitative PCR. Strain frequencies were estimated by quantitative PCR and densitometry (Baganz *et al*, 1998). Genomic DNA was extracted with the Nucleon MiY kit (Amersham). PCR reactions with primers 6F, 6R, 7R and 16F, 16R, 7bf were used to assess the frequency of the VI/VIIt and VII/XVIt translocations, respectively. For each experiment, all reactions were carried out simultaneously with the same Master Mix. PCR conditions were 94 °4 min–(94 °45″–57 °45″–72 °1 min) × 35–72 °5 min. PCR products were run on 1.5% agarose in TAE gels stained with 0.2 μg/ml ethidium bromide. Densitometry was performed using Quantity One software (Bio-Rad). The strain frequencies were then interpolated from calibration curves constructed from strain mixtures of known frequencies. Estimations from three PCR

Table 2 | Primers used for insertion of the *loxP* sites in the genome by PCR-mediated gene replacement, and for the verification of the rearrangements by analytical PCR

Primers for disruption cassettes

Primer names	Primer sequences
VI/VIIt translocation	
YFR0607	F: atgattactagtgcttaaaccttgttattcatgcctcagctgaagcttcgtacgc
	R: aacctaacaaggttacttcatttttcccgaagagcaatacgcataggccactagtggatc
YGR2122	F: actgtaataaagagtaaaaacaacaacaaagacaatcgagctgaagcttcgtacgc
	R: gtgtttaaacaaatttcggaagcaatggagcatcaggccactagtggatc
VII/XVIt translocation	
YGR188189	F: ccgcactttatattttaacgtttgatggttttctatcttggcagcgcagctgaagcttcgtacg
	R: ctttttattattaaggggtagccttcatacaaaggaaatttatgtcatcgcataggccactagtggatc
YPL116103	F: cgctgcttctataacacttgtttttgacatgacgataagtgtagtcagctgaagcttcgtacgc
	R: ataaattgattctaagtacaggaagtatcaagggggggggttgacgcataggccactagtggatc

Primers for analytical PCR

Primer names	Primer sequences	Primer pairs	Fragment sizes (bp)		
			Wild-type size	With no translocations	With translocations
VI/VIIt translocation					
Ck6F	cgtcgtccacctatttgag	Ck6F/Ck6R	1,046	765	–
Ck6R	atccaatagctgaagaacgtc	Ck7F/Ck7R	1,060	605	–
Ck7F	acatcagagacttgggcatc	Ck6F/Ck7R	–	–	865
Ck7R	agctgtgctcgatgttgtg	Ck7F/Ck6R	–	–	495
VII/XVIt translocation					
Ck7bF	gttggcacaagttatggagtc	Ck7bF/Ck7bR	1,259	667	–
Ck7bR	cgtagctttactagccttgc	Ck16F/Ck16R	914	508	–
Ck16F	ttattcccctcatatattcgg	Ck7bF/Ck16F	–	–	634
Ck16R	ttattatgcaagttgtgtaggc	Ck16R/Ck7bR	–	–	560

reactions per sample were averaged to give the final value. Selection coefficients were calculated as for the batch experiment, except that each strain's frequency was compared with the sum of the frequencies of the two competing strains.

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