

## Supporting Online Material for D. Greig *et al.* 1076374, published 29 November 2002

### Materials and Methods

*Strains.* All *S. cerevisiae* strains were isogenic with Y55 (*S1*). All *S. paradoxus* strains were isogenic with N17 (*S2*). Homothallic diploid *S. cerevisiae* strains YDG 96 (*lys2/lys2*) and YDG 97 (*ura3/ura3*) were starved to induce meiosis and crossed to homothallic diploid *S. paradoxus* strains N17-71 (*ura3/ura3*) and N17-64 (*lys2/lys2*) respectively, producing two heterozygous prototrophic F<sub>1</sub> hybrids, YDG124 and YDG125. Strains in groups A and C were derived from YDG124 and YDG125 respectively and were all *ura3*, *LYS2*. Strains in groups B and D were also derived from YDG124 and YDG125 respectively but were all *URA3*, *lys2*. These auxotrophic markers make it possible to cross strains from group A with either strains from group B or group D, and strains from group C with strains from groups B and D. Five pairs of strains with high individual fertility were selected for crossing, subject to these constraints. The strains were crossed, sporulated and their tetrads dissected as described above. Spores that germinated, autofertilized (producing F<sub>4</sub> hybrids) and were auxotrophic were collected. Strains were germinated and grown on YEFD unless a minimal synthetic medium with appropriate added amino acids was required for selection or screening of particular genotypes. Strains were sporulated on potassium acetate medium. All media were prepared according to standard recipes (*S3*).

*Fertility measurement.* The fertility of strains was defined as the percentage viable gametes they produced, measured by standard tetrad dissection. All fertility analyses were performed with arcsine transformed data, which yield asymmetric *t*-distribution-based confidence intervals (*S4*).

*Karyotype assays.* Genepairs primers (Research Genetics, Huntsville, AL) were selected to amplify an Open Reading Frame at each end of every *S. cerevisiae* chromosome by standard Polymerase Chain Reaction. Annealing temperatures were optimized so that *S. cerevisiae*, but not *S. paradoxus*, template DNA would yield product. Primers that were not sufficiently specific were replaced with alternatives. We used the following sets to determine the karyotypes of the 38 sampled F<sub>2</sub> hybrids (annealing temperatures indicated in parentheses): YAL059W (59°C), YAR033W (64°C), YBL092W (63°C), YBR282W (67.2°C), YCL058C (61°C), YCR090C (61°C), YDL228C (64°C), YDR529C (61°C), YEL067C (61°C), YER180C (61°C), YFL046W (61°C), YFR047C (67°C), YGL250W (61°C), YGR280C (61°C), YHL037C (66°C), YHR213W (61°C), YIL168W (61°C), YIR032C (61°C), YJL215C (61°C), YJR146W (61°C), iYKL225W (52°C), YKR100C (61°C), iYLL067C-0 (52°C), iYLR466W (52°C), YML122C (67°C), YMR314W (62°C), YNL319W (64°C), YNR068C (61°C), iYOL166C (60°C), YOR383C (61°C), YPL266W (63°C), YPR177C (68.5°C). The resulting data are presented in Table S1, and a sample gel is shown in Fig. S1.

Measurement of tetrasomy for chromosomes I, II, and VIII by pulsed field gel electrophoresis was performed as previously described (*S5*). Three hybrids grew so poorly that their chromosomes could not be scored reliably—they were removed from the analysis. Tetrasomy of other chromosomes could be detected by their extra-bright bands, but we found scoring these to be subjective so we restricted the analysis to the three distinct chromosomes. Sample images of the pulsed-field gels are shown in Fig. S2.

*Fitness assays.* Fitness was calculated as the ratio of Malthusian parameters determined by direct competition against a common genetically marked competitor (*S6*). The frequency of each competitor at the beginning and end of competition was determined by flow cytometry, using gal-induced green fluorescent protein (GFP) as a marker. The Y55 strain background does not give strong gal-induction so its *gal3* allele was replaced with the *GAL3* gene from plasmid pJH618 (provided by Neil Hunter) by two-step gene replacement. Correct replacement was confirmed by tetrad analysis. It was then transformed with the *XhoI* fragment of pDG86 (this study), which transplaces *LYS2* with a GFP gene under the control of a *GALI* promotor, producing strain YDG152. This strain was crossed to produce a GFP-marked prototrophic diploid standard competitor.

*Statistical analyses.* Significance testing for sources of variation in fertility among  $F_2$ ,  $F_3$ , and  $F_4$  hybrids was determined by ANOVA. The contribution of genic interactions in  $F_2$  hybrid fertility was estimated by the difference in a nested ANOVA, with genotype nested within block..

**Table S1. Karyotype of F<sub>2</sub> hybrids**

The column heads indicate the chromosome ends (l for Left Arm and r for Right Arm), labeled in Arabic rather than Roman numerals and ranked from smallest chromosome (left hand side of table) to largest chromosome (right hand side of table). The rows indicate the hybrid strain designation. A “1” in a cell indicates a positive PCR reaction, a “0” indicates a negative reaction. For example, hybrid **a19** has a “1” in column **5l** and a “0” in column **5r**. It thus contained the left end of chromosome V but lacked the right end. Thus it is a recombinant for this chromosome, and the yellow background in the cell indicates this. A corresponding example gel is shown in Figure S1.

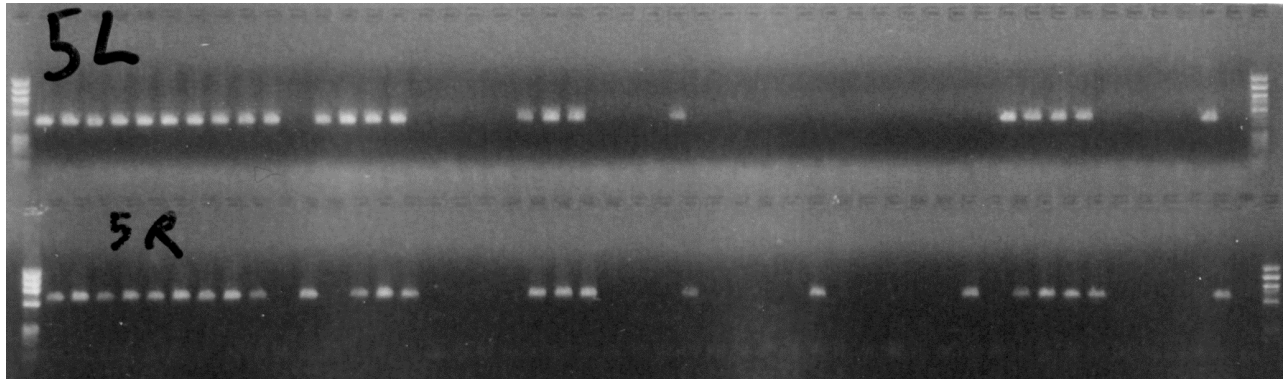
Hybrid designation	CHROMOSOME END																															
	1l	1r	6l	6r	3l	3r	8l	8r	9l	9r	5l	5r	10l	10r	2l	2r	11l	11r	16l	16r	14l	14r	13l	13r	15l	15r	12l	12r	7l	7r	4r	4l
a10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
a12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
a13	1	1	0	0	1	1	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0	1	1	1	1	0	0	1	0	1	1	1
a14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
a16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
a17	0	0	1	1	1	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a18	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a19	1	1	1	1	1	1	1	1	0	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0
a20	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	1	1	1	0
b13	0	1	0	0	1	1	1	1	1	1	0	0	0	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0
b14	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b15	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	0	0	1
b16	0	0	1	1	1	1	1	0	0	0	1	1	1	1	1	0	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1
b17	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
b18	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
b19	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
b20	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
b3	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
b4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
b5	0	0	1	1	1	1	1	0	0	0	1	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0
c1	1	1	0	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
c4	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0	1	0	1	0	1	1	1	1	0	1	1	0	0	0	0	0	0
c5	1	0	1	1	1	1	1	1	0	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0
c6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
c7	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
c9	1	1	1	1	1	1	0	1	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c13	1	1	1	1	1	1	0	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1
c14	1	1	1	1	1	1	0	0	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0
c18	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
c19	1	1	1	0	1	1	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
d11	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
d12	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
d13	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	0	0	0
d14	1	1	0	1	1	1	1	1	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
d15	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1
d16	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
d17	0	0	1	1	1	1	0	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0
d18	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0
<i>S. cerevisiae</i> control	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. paradoxus</i> control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Notes:  
 \*1\* indicates marker present, \*0\* indicates marker absent  
 chromosomes are ranked from left to right by ascandin *S. cerevisiae* genetic size  
 yellow background indicates a crossover between *S. cerevisiae* and *S. paradoxus*  
 Hybrids a11, a15, d2, d3, d4, d5, d19 and d20 were removed from the PCR analysis so that the karyotype data set would match the fitness data set. Including these strains does not affect the conclusions drawn.

### Figure S1. Sample PCR gel

This gel shows a sample PCR assay for left (upper row) and right (lower row) ends of *S. cerevisiae* chromosome V. The 2<sup>nd</sup> to the 49<sup>th</sup> lanes contain PCR products using the following strains as templates, from left to right: a10, a11, a12, a13, a14, a15, a16, a17, a18, a19, a20, b13, b14, b15, b16, b17, b18, b19, b20, b3, b4, b5, c1, c4, c5, c6, c7, c9, c13, c14, c18, c19, d11, d12, d13, d14, d15, d16, d17, d18, d19, d20, d2, d3, d4, d5, *S. cerevisiae* control, *S. paradoxus* control. Hybrids a11, a15, d2, d3, d4, d5, d19 and d20 were removed from the PCR analysis so that the karyotype data set would match the fitness data set. Including these strains does not affect the conclusions drawn.

As an example of how to interpret the gel, examine lane 12. This corresponds to hybrid strain **a19**. There is a band in the upper row, but not in the lower row. This indicates that the left end of chromosome V was present, but not the right end. This example matches the example in Table S1.

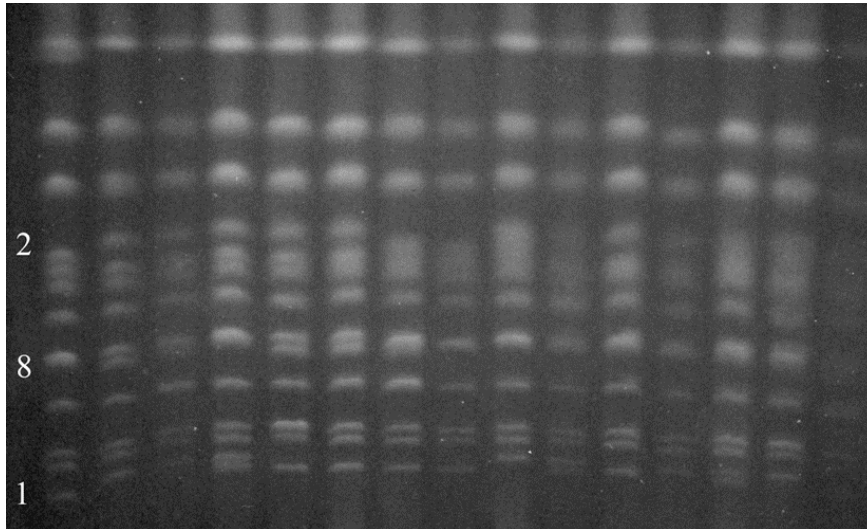


**Figure S2. Sample pulsed field gels**

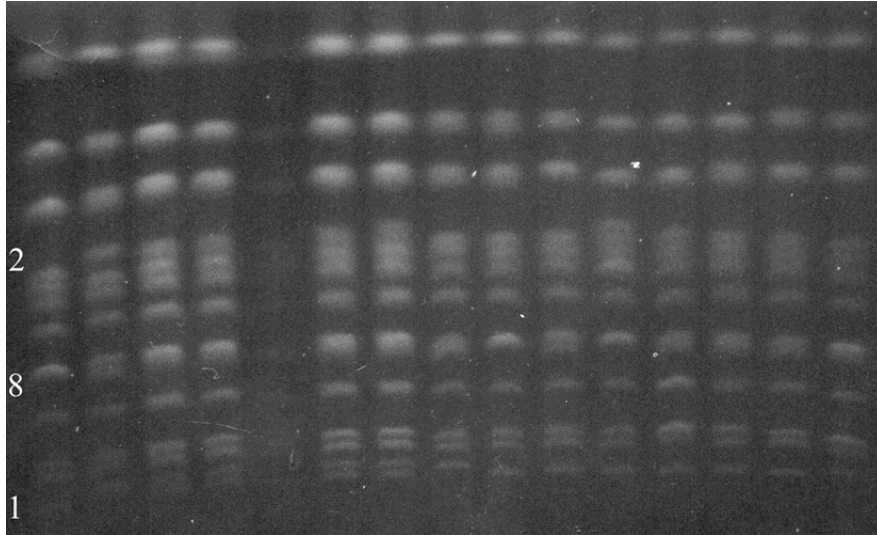
These three images show examples of the Clamped Homogenous Electric Field (CHEF) pulsed field gels used in the karyotype analysis. The 3 chromosomes that can be identified clearly as either *S. cerevisiae* or *S. paradoxus* are indicated with the numerals 1, 2, and 8 for chromosomes I, II and VIII, respectively.

As an example of how to interpret the gels look at CHEF1, below. Examine the lowest band, which is chromosome I (marked 1 on gel). This band in lane 1 (*S. cerevisiae*) is lower than the lowest band in lane 2 (*S. paradoxus*). Lane 4 is very clear in this example, and both bands can be clearly identified, indicating that this strain (hybrid **a14**) contains chromosome I from both species, and is thus tetrasomic for this chromosome.

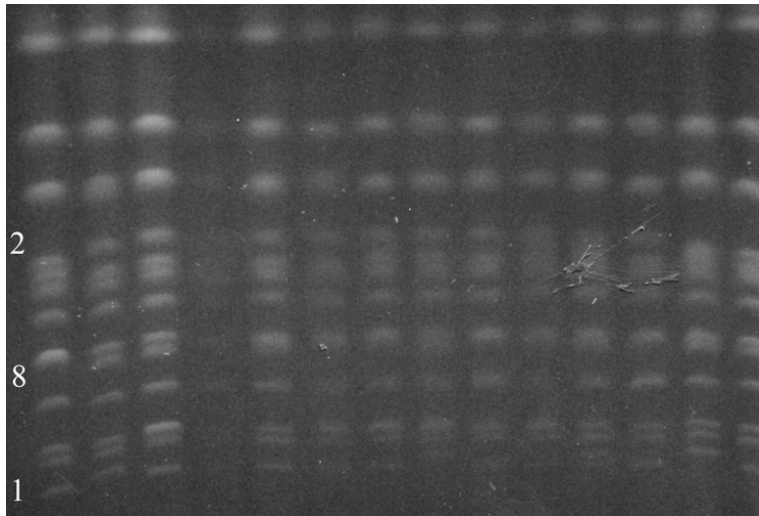
CHEF1 Lane 1 contains *S. cerevisiae* control, lane 2 contains *S. paradoxus* control, lanes 3 to 15 contain, from left to right, a10, a12, a13, a14, a16, a17, a18, a19, a20, b13, b14, b15, b16



CHEF2 Lane 1 contains *S. cerevisiae* control, lane 2 contains *S. paradoxus* control, lanes 3 to 15 contain, from left to right, b17, b18, b19, b20, b3, b4, b5, c1, c4, c5, c6, c7, c9



CHEF3 Lane 1 contains *S. cerevisiae* control, lane 2 contains *S. paradoxus* control, lanes 3 to 14 contain, from left to right, c13, c14, c18, c19, d11, d12, d13, d14, d15, d16, d17, d18



## References

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